DEVELOPMENT OF ATOMIC ENERGY IN BRITAIN

THE Atomic Energy Authority Bill, the second reading of which in the House of Commons on November 18 was moved by Sir David Eccles, amends two provisions in the Atomic Energy Authority Act, 1954. The first raises from ten to fifteen the maximum number of 'members' of the Authority, and the second enables the Authority to include in its pension scheme those of its staff who are transferred to the employment of the National Institute for Research in Nuclear Science. The reasons for these amendments were explained by Sir David and also, on December 3, by Lord Hailsham, when as Lord Privy Seal and Minister for Science he moved the second reading in the House of Lords.

Sir David Eccles said that the first clause of the Bill sought to recognize the growing complexity of the Authority's work and to restore a measure of flexibility for the future in the composition of the governing body while preserving the balance between full-time and part-time members. Since 1954, the staff of the Authority had increased from 17,000 to 37,000, and the Industrial Group alone now employs more than 18,000, a figure expected to exceed 23,000 by 1961–62. Between 1956 and 1959 the Authority increased its staff scientists and engineers by 71 per cent, and the figure of 4,220 is expected to increase by 42 per cent by 1962. At present, some 63 per cent are engaged on research and development, and the industrial consortia themselves are believed to employ some 4,000 men on reactor design and development.

The immediate requirements arise partly out of the recent decision to split the Industrial Group into a Development and Engineering Group, in charge of Sir William Cook, already a full-time member of the Authority, and a Production Group, of which Sir Leonard Owen had been designated head. It was desirable that Sir Leonard Cook, too, should be a full-time member. Further, it was desired to retain the services, as a part-time member, of Sir John Cockcroft, now Master of Churchill College, Cambridge, whose place as member for research had been taken by Sir William Penney. While at present the need is only for two further places, it was considered better to provide for a maximum of fifteen, rather than the twelve immediately required.

As regards the second clause, Sir David Eccles explained that the National Institute for Research in Nuclear Science, established to help the universities and other learned bodies participate in fundamental research in nuclear physics, is growing fast, and its staff is largely interchanged with the staff of the Authority. At present the Authority has no power to admit the staff of the Institute to its superannuation schemes if they so desire. Lord Hailsham made it clear that this permissive power would apply to both temporary and permanent exchange.

Although the Bill was generally welcomed in both Houses, there was considerable discussion on particular points in the House of Commons. Mr. A. Greenwood, who opened the debate, welcomed it as the first of any substance on the Authority since 1954; but while stressing the importance of part-time membership of the Authority as facilitating the participation in its work of distinguished men of science and industry, he expressed some misgivings as to consistency in the higher planning of the Authority's work. He recognized the need for flexibility, but although the Select Committee on Estimates had endorsed both the decision to split the Industrial Group and to restore executive responsibility to the technical members of the Authority in spite of the recommendations of the Fleck Committee, Mr. Greenwood said he is not entirely reassured. Stressing the importance of comparable conditions of employment in the Authority and at the National Institute for Research into Nuclear Science, he pointed out that in 1958 the Industrial Group had vacancies for 258 university graduates. The Authority had offered posts to 422 graduates, but only 160 accepted the appointments offered.

Mr. A. Albu, who also supported the Bill, urged the increase of Government expenditure on fundamental research as likely to benefit the industry, and referred to anxiety, particularly in the Isotopes Division, due to the frustration by Treasury control of the efforts of the scientists. Mr. Albu asserted that in the past two years many heads of departments in the Authority had resigned because of frustration, and he thought that this might bear on difficulties experienced in recruiting senior staff. The main administrative difficulty at present, he said, is lack of a sense of direction, and this is directly attributable to the retardation in the commercial development of reactors in consequence of the change in the energy position in Great Britain. Mr. Albu himself suggested concentration on a small type of reactor suitable both as a marine reactor and as a small land-based power station, which he thought should have a considerable market and assist and benefit the under-developed countries. He also thought that we should go much further in co-operating with other countries in Europe, but he did not mention that Britain's only opportunity with Euratom is now in the 1,000-megawatt nuclear programme which at the outside may be added to the 2,000-megawatts of nuclear plant which is being commissioned by 1965.

This plea for clearer understanding of the economics of nuclear energy was supported by Mr. H. Davies, who asked for a further opportunity for a constructive debate on nuclear energy and referred, in particular, to the need to release more information from the restrictions of secrecy. Another plea for a further debate came from Mr. R. Mason, who, questioning whether the decision to develop gas-cooled graphite-moderated reactors had been wise, expressed concern as to the shortage of scientists,

technicians and engineers and, in particular, regarding the loss of so many highly qualified scientists abroad. He thought we should make a serious attempt to ascertain why so many good scientists, technologists and engineers, who accepted research scholarships in the United States and Canada, failed to return. The relations with industry also required consideration, and in particular we should find some way of using to a greater extent the consortia and their experienced staff.

In replying on the debate, Sir David Eccles assured the House that there is no intention of stopping work on the manufacture and sale of isotopes. The recommendations of the Select Committee on Estimates on the relations between the Authority and industry had been accepted by the Authority. Relations in reactor technology are already close, and the observations of the Committee on the manufacture of fuel elements are receiving attention. Referring to recruitment, Sir David said that the senior professional staff had increased from 377 on March 31, 1958, to 511 at the end of September; for scientists and engineers, the figures were 6,900 and 8,600, while the senior administrative staff had increased from 56 to 93. The Bill was passed without amendment at the Committee stage at the third reading on November 25.

The debates clearly lent much support to the findings of the Select Committee on Estimates in its Fifth Report for the Session 1958–59; but they should also stimulate that wider thinking about the development of nuclear energy in the changed context of high-level stocks of solid fuel, even if in Great Britain there are not the low fuel prices that, with large stocks, have caused Europe's need of nuclear energy to recede into the 1970's. The only way to sell nuclear power is manifestly to sell it cheap, and the Minister for Science should note the strong demand for regular debates on nuclear energy for the further reason that much constructive thinking is still needed on the relations between the Authority and industry and with Euratom. What is being done at Winfrith Heath will obviously be affected by the duplication and waste already apparent in the research programmes of the six member countries of Euratom. The new outlook for nuclear power inevitably forces on Great Britain the need for greater watchfulness to avoid waste of resources and manpower in its development, alike from the competitive point of view and from that of the efficient use of our own limited resources, with which the Minister for Science is intimately concerned. Meanwhile, it should be noted that, since the debate, it has been announced in London, on December 4, that teams of scientists from Great Britain and Euratom countries are to co-operate in research on controlled thermonuclear reactors, fast breeder reactors and advanced gas-cooled reactors. It is hoped that the new agreements, besides eliminating wasteful overlapping in research projects, may lead to more efficient use of scientific man-power, as well as to exchange of information, co-ordination in forward planning by the British and Euratom teams, and interchange of personnel between Britain and the Continent.

A RATIONALE FOR BEHAVIOUR

Biological and Biochemical Bases of Behavior
Edited by Harry F. Harlow and Clinton N. Woolsey. Pp. xx+476. (Madison, Wisc.: University of Wisconsin Press, 1958.) 8 dollars.

ACCORDING to the preface of this book, the symposium which it represents derived from the activities of a sub-committee to explore the relationship between the biochemical and biological sciences and mental health, and its purpose was to correlate behaviour with results obtained from other biological sciences: anatomy, embryology, physiology, pharmacology and biochemistry. In the present state of knowledge, this might appear to be somewhat ambitious and the biochemistry of behaviour is, to say the least, a study for the future. Nevertheless, the rational basis of behaviour will be found only by the persistent study of the related basic sciences.

It is perhaps unfortunate that, for reasons of economy, the comments of those taking part and the general discussion have been omitted from the book, since it might be expected that this part of the symposium would produce more integration between different fields. As it is, the book consists of a number of isolated contributions, with only one common link; namely, that each represents some aspect of the study of behaviour. When compared with other similar publications of which there has been a spate in recent years, this book is perhaps unique in that the major contribution is provided by experimental psychology. Both the opening and closing chapters are written by psychologists (Harlow and Hebb) and both emphasize the contributions of psychological method to the study of behaviour. They also stress that the psychologist must turn to other disciplines for an explanation of the mechanisms underlying the phenomena he is studying, an important step which not all have taken. It is not surprising, therefore, that more than one-third of the book is concerned with the relation between function, in terms of behaviour, and structure; this is illustrated by the effects of lesions in the brain on various aspects of behaviour, for example, sensory discrimination. Such studies, examples of which are too numerous to quote, lend themselves most readily to the application of psychological methods.

Neurophysiological contributions to behaviour are represented by those of Magoun and Jasper, who discuss the brain stem reticular activating system in relation to sleep and wakefulness and the role of diffuse projection systems in integrative processes, such as conditioning. Lilly describes the plotting of a 'motor' map of the cerebral cortex from observations of movements produced by localized electrical stimulation utilizing 610 implanted electrodes! Olds deals with the relationship between electrical events and behaviour in self-stimulation studies, the full implications of which have yet to be clarified. There is one purely anatomical paper (Woolsey).

There are three biochemical contributions to this book. Dr. Tower effectively summarizes the present state of neurochemistry, emphasizing the effects of biochemical lesions, for example, phenylketonuria, on cerebral function. He concludes with remarks that the neurochemical substrates of cerebral activity must, at some stage, be incorporated into the neurophysiological and behavioural structure of the central nervous system. Rosenzweig, Krech and Bennett

have, in fact, made an attempt to correlate neurochemistry and behaviour, and theirs is a rare study involving collaboration between biochemists and behavioural scientists. They have investigated the relationship between acetylcholine metabolism, measured by cholinesterase activity, and the maze behaviour of rats. This work is extremely interesting, and, if it does nothing else, it re-emphasizes the importance of biochemical studies on small areas of brain tissue. Indeed, any study which could throw light on the changes in the central nervous system accompanying the learning process is more than worthwhile. However, as Tower points out, not only is the role of acetylcholine in the brain equivocal but also cholinesterase activity is a doubtful measure of its metabolism.

Beach discusses sexual behaviour and its modification by hormonal and neuronal mechanisms. It is of interest to recall that profound changes in primitive behaviour of this type have been known to be affected by different chemical structures for some time. Recent advantages in the elucidation of the structure of hormones and their chemical synthesis should provide an even sounder base-line for future study.

A book of this nature usually provides a fund of vogue words; "interdisciplinary" and "major breakthrough" have been met before but "ideational" is new to us. As is inevitable in publications of this nature, much of the material has been published elsewhere, either before or since the symposium was held, while other contributions represent entirely new work. This does not detract from the value of the book nor does the fact that it is now three years since the symposium took place. It is a useful guide to current thought and knowledge and should be read by all interested in the study of behaviour. The book is provided with an adequate subject index.

G. B. Ansell
P. B. Bradley

CRYSTALLOGRAPHER'S TOOL-CHEST OF MATHEMATICS

International Tables for X-ray Crystallography

Edited by John S. Kasper and Kathleen Lonsdale. Vol. 2: Mathematical Tables. Pp. xviii+444. (Birmingham: The Kynoch Press, 1959.) 115*s*.

THESE tables form the second instalment of the complete revision and expansion of the original "Internationale Tabellen" of 1935. The history of those tables and of the present project have been described by Ewald in his review (Ewald, P. P., *Nature*, 171, 944; 1953) of Vol. 1 of the present tables. Vol. 2 has been compiled with the object of including all the formulæ and mathematical tables that a crystallographer may be reasonably expected to require. They contain no data that depend on physical measurement; such data will appear in Vol. 3.

Perhaps the most significant innovation in these tables is the inclusion of a 90-page section on mathematical methods. This covers algebra, trigonometry (plane and spherical), calculus (integral and differential), vector and tensor analysis, Fourier-transform theory and statistics, and comprises definitions, theorems, methods of attack, and examples of practical applications to crystal structure analysis. Here one can find readily, for example, how the characteristic values and vectors of a matrix may be calculated; what methods are available for the numerical solution of transcendental and other 'insoluble' equations, and what are their relative merits and particular applications; what are the general properties of groups; how the Fourier transform of a discontinuous function may be expressed in terms of the discontinuities; how to apply a statistical significance test; over what range of values of x is $\sin x$ equal to $x - 1/6\ x^3$ to within 0·001; how a given number may be approximated to a rational fraction by the use of continued fractions. There are many references to standards texts and to special articles. It is surprising, however, that interpolation formulæ are not treated, since these should be within easy reach of anyone who uses tables, and are moreover useful whenever the question of the most suitable interval for the tabulation of mathematical functions or of experimental observations is under consideration.

Under the heading "Crystal Geometry" there are definitions and formulæ relating to direct and reciprocal lattices, angles between planes, twinning conditions, and quadratic forms. A section on diffraction geometry deals with all the well-known techniques for recording X-ray diffraction patterns; their particular characteristics and applications are listed and formulæ and tables relevant to each method are given. The accuracy of measurement of lattice parameters is treated in considerable detail. "The Physics of Diffraction Method" covers formulæ for the intensity of diffraction by a crystal, and the corrections for temperature effects, absorption and extinction. Under "Fourier Synthesis and Structure Factors" appear formulæ for the various types of synthesis that are in use, and methods of structure refinement are fully treated. There is also a survey of digital and analogue methods of numerical Fourier synthesis.

Further topics are the close packing of spheres in various space groups, statistical tests on intensity data for the detection of symmetry elements, and inequalities between structure factors that may give information about their phases. (The *probable* relations between phases are not included, however.) Finally, there are some useful sets of trigonometrical tables (tailored for crystallographers), and a glossary of crystallographic terms in English, French, German, Spanish and Russian, in rather more detail than that in Vol. 1.

The criteria on which such tables are to be judged are those of comprehensiveness, ease of use, and comprehensibility. On the first and second scores it is almost impossible to find any fault. The compilers took the view that nothing of potential application should be left out, since few crystallographers have a perfect memory for formulæ, and most prefer not to spend time working them out or searching textbooks. A very full and systematic contents list makes all material easily accessible, and renders an alphabetical index unnecessary. On the third score it is again difficult to find fault, but one wonders whether perhaps mathematical elegance and logical development have occasionally been given first place rather than ease of reference; for example, the introduction to the section on "Fourier Theory" (surely a slight misnomer because Fourier also had a theory of heat conduction) might well scare the weaker mathematician into thinking that he has come to the

wrong book. But this criticism does not apply generally.

The layout and printing are of excellent quality, and the binding is pleasing in appearance and made for hard use. The price is in line with that of any first-class text-book of to-day; if it saves a laboratory a total of five days of research-worker time or one hour of computer time it will have yielded a handsome profit.

A. R. Stokes

THOUGHTS ON COMMUNICATION

The British Association Granada Lectures

Science and Communication. By Sir Edward Appleton. Television and Politics. By Edward R. Murrow. Dons or Crooners? By Sir Eric Ashby. (Three Lectures given in Guildhall, London, in October 1959, on the subject of "Communication in the Modern World".) Pp. 111. (London: Granada TV Network, Ltd., 1959.) 3*s*. 6*d*.

THESE three lectures were organized by the British Association and sponsored by Granada TV Network, Ltd. They were delivered in Guildhall, telecast later in a considerably shortened form, and now appear in print. This immediately presents one with the problem of how to treat them: as lectures, television or essays? Not having seen them on television, I will ignore this aspect.

The general title of the three lectures is "Communication in the Modern World". None of the lectures conflicts with this title, but they are related only formally.

The first lecture, by Sir Edward Appleton on "Science and Communication", gives a lucid account of modern communications, but says little that is new. One expects a few original ideas in a 'prestige' lecture of this sort. He might well have extended the section where he speculated about future developments. From a man so eminent in the field of communications, this would have been far more interesting than a summary of our present knowledge, as already several good ones exist.

Dr. Edward R. Murrow gave the second lecture on "Television and Politics". He throws out ideas, wittily recounts numerous telepolitical anecdotes and very wisely draws the final conclusion: "In the area of political television we are all ignorant". Another point he makes, rather more tentatively, is that it is personalities that come across, not ideas. For example, Vice-President Nixon during his election campaign was accused of accepting too many presents. He appeared on television and won overwhelming support from the public by refusing to give up the one present he admitted to receiving—a little dog—because "the kids love that dog". Similarly, Dr. Murrow ascribes McCarthy's rise largely to his flair for publicity on television, and he suggests that Stevenson would have done better in a non-television election where he was not up against Eisenhower's homely personality. But—"It is a limited medium . . . it can arouse curiosity . . . entertain . . . sell goods. But it can't reason . . ."

Sir Eric Ashby, in his lecture "Dons or Crooners?", is the only one really to put forward a thesis. It is this: The ordinary man does not feel that he really belongs to society, as he did, say, in the Middle Ages. This is because he is unable to understand the science on which it is based. Therefore, he must be given some understanding of science, and this should be done by emphasizing the importance of technique in science, for the ordinary man is a craftsman and he appreciates craftsmanship. This is an excellent plan so long as it is not regarded as the only one. But Sir Eric is very patronizing about the ordinary man. He is called Ron Blossom and he lives in Doris Grove, Birmingham. His response to a dramatic scientific discovery is to say "Cripes". One thing no one can afford to do if he wants to put an idea across without antagonizing the recipient is to patronize him. Surprisingly for the author of "Technology and the Academics", Sir Eric says: "The interpretation of science to the likes of you is no problem. You have the Third Programme and Pelicans . . ."; 'you' in this case being a 'Third Programme audience'. Now it is only true that the interpretation of science to highly intelligent people presents no problems if the people are interested. The trouble is that a great many of them are not. Where there is a will there may be a way. But it does not follow that where there is a way there is a will.

B. Silcock

DESCRIPTIVE ASTRONOMY

Elementary Astronomy

By Prof. Otto Struve, Beverly Lynds and Helen Pillans. Pp. vii+396. (London and New York: Oxford University Press, 1959.) 55*s*. net.

THIS is a text-book based on a new approach to the study of astronomy. Many elementary texts have considered primarily the historical and purely factual descriptive aspects of astronomy. Recently, there has been felt a growing need for a course emphasizing the physical basis of astronomy, using at most the simplest mathematics to perform calculations illustrative of the physical principles involved, but still including enough descriptive material to give the student a basic knowledge of modern astronomy. This book aims at meeting this need, and it fulfils its intentions admirably. The subjects are treated in more or less conventional order, starting with the Earth, Moon and planets, followed by the Sun, the stars and star clusters, and ending (apart from a chapter on instruments) with galaxies, but the book has quite a different tone compared to older texts. Rarely has a volume intended for serious students been so profusely illustrated, excelling most of the avowedly popular books. Still more rarely has any general text-book contained so many illustrations from the recent periodical literature. The senior author has in the past attained a reputation for his descriptive articles no less than for his research work, and this book fully maintains this reputation.

Astronomy, in common with all other sciences, is developing very rapidly, and no one can be an expert in all its branches. Yet the whole of this book is surprisingly up to date, a feature which will commend it to teachers of astronomy. Most of the book will be intelligible to amateur astronomers, and it should find a place on the shelves of every general library with an astronomy section. The authors state in the preface that their book is not intended to replace the many excellent text-books of descriptive astronomy now in use. The reviewer is confident that it will indeed become the leading introductory text on its subject.

R. H. Garstang

Recent Advances in Atmospheric Electricity

Proceedings of the Second Conference on Atmospheric Electricity, held at Portsmouth, New Hampshire, May 20–23, 1958. Edited by L. G. Smith. (Sponsored by Aerophysics Laboratory, Geophysics Research Directorate, Air Force Cambridge Research Center, Air Research and Development Command.) Pp. xv+631. (London and New York: Pergamon Press, 1958.) 120*s*. net.

THIS book reproduces the fifty or so papers that were presented at the conference named in the sub-title. They are arranged under three sub-divisions: fair-weather electricity, thunderstorm electricity and the lightning discharge. In some cases a résumé of the discussions following a paper is included. There does not appear to have been any attempt at making a selection of the more important contributions for publication and in consequence the value of individual papers varies widely. It is difficult to find out from such a varied assortment of material how much of it does really represent recent advances in the subject; some of the papers describe work that is already well known, while others are more in the nature of interim reports.

It is significant that a large part of the work described is devoted to atmospheric electrical investigations in the upper air by means of aircraft measurements and radio sondes. Another significant trend is the use of radar in studying thunderstorm precipitation and lightning.

Interested people who were unable to attend the conference will welcome the opportunity of learning what went on there, and there is no doubt that the active worker in the subject can find a great deal of interest to him in many of the papers reproduced in the book. But it is very doubtful whether a book as expensive as this, beautifully produced though it is, will attract many individual purchasers.

F. J. SCRASE

Principles of Organic Chemistry

By T. A. Geissman. Pp. viii+635. (San Francisco: W. H. Freeman and Company; London: Bailey Bros. and Swinfen, Ltd., 1959.) 7 dollars; 56*s*. 6*d*.

THE author has written an elementary text-book of organic chemistry which includes most of the material required by a first-year university student, but which has its emphasis on important functional groups and on general types of organic reactions. This approach leads to obvious omissions in a book of such moderate size, but Prof. Geissman holds very reasonably that the student's time is initially better spent in the study of principles than in the acquisition of general information. The last sixty pages, however, are largely descriptive and deal with structurally more complicated molecules, many of which are biologically important. Thus the discussion of the basic principles of the subject has been complemented by some of its major achievements and applications. In this way, the student is shown some of the scope of organic chemistry.

Prof. Geissman has, on the whole, succeeded in his objects. The presentation and printing of the book are both good, except that some of the diagrams of molecular models are confusing and that a few errors in formulæ may be misleading to students. Perhaps the allocation of space to various topics is a little unbalanced. For example, more room is given to aromatic electrophilic substitution than to stereochemistry. Nevertheless, this book can be enthusiastically recommended as an interesting and stimulating introduction to organic chemistry.

C. B. REESE

Introducing Astronomy

By J. B. Sidgwick. Pp. 259. (London: Faber and Faber, Ltd., 1959.) 6*s*. net, paper bound.

THIS book is a fairly drastic revision of the same author's "Astronomy for Night Watchers" and, as such, is aimed at promoting an interest in astronomy in the layman; no knowledge of mathematics or physics being presumed. It is divided into two roughly equal parts. After two introductory chapters, the first part consists of an elementary outline of descriptive astronomy, taking the usual route outwards from the Sun to extra-galactic nebulæ; followed by simple explanations of some astronomical instruments. The second part is almost entirely taken up with a description and sketch of each constellation in turn, mentioning such objects of especial interest as may be observed with binoculars. These artificial divisions may be a disadvantage from an observer's point of view but allow the inclusion of a brief history and mythology of the constellations.

Mr. Sidgwick writes with an easy style and has succeeded in producing a very readable and balanced introduction to astronomy with very few errors, none of which seriously detracts from its value. Definitions are introduced as necessary and there are numerous cross-references, so the beginner should encounter few, if any, stumbling-blocks. The reader whose new interest in this old science has been so ably fostered is likely to want to know more. It seems a pity, therefore, that space has not been spared for even a short bibliography.

B. R. LEATON

The Birds of the British Isles

By Dr. David Armitage Bannerman. Vol. 8: Phalacrocoracidae, Sulidae, Fregatidae, Procellariidae, Diomedeidae, Podicipedidae, Gaviidae, Columbidae, Pteroclididae. Pp. x+400+26 plates. (Edinburgh and London: Oliver and Boyd, Ltd., 1959.) 63*s*. net.

THE eighth of Dr. Bannerman's impressive volumes is largely concerned with sea-birds—the cormorants, the gannet and the petrels. The magnificent frigate-bird is included on the strength of its sole recorded appearance, in 1953, and the black-browed albatross in view of two certain and a few doubtful records; with these, as also with the rarer petrels, the author follows his practice of giving full information from the regions where the birds more truly belong. Then come the grebes, the divers, the pigeons and, finally, Pallas's sandgrouse. The remarkable spread of the collared turtle-dove, unknown in Britain until the present decade, is duly chronicled; so also are the extraordinary occasional visitations of the sandgrouse from the Aral-Caspian region. To an increasing extent Dr. Bannerman has included contributions from other writers. Thus the whole chapter on the gannet is by Mr. George Waterston and those on two of the exotic petrels are by Dr. Alexander Wetmore, while various other chapters have sections by ornithologists with special knowledge of particular aspects.

LANDSBOROUGH THOMSON

MAGNETOTROPISM: A NEW PLANT-GROWTH RESPONSE

By Prof. L. J. AUDUS

Botany Department, Bedford College, London

TWO major theories of graviperception now stand opposed. One due to Brauner[1] is based on the so-called geoelectric effect. Apart from the uncertainty cast on this whole phenomenon by recent investigations[2], I have long felt that the forces involved were utterly inadequate to cause the extremely rapid and extensive changes in hormone concentration required by the observed rates of growth response. In the second and classical statolith theory, cell particles having a density different from that of the surrounding cytoplasm move under gravity to one side of the responding cell, thereby evoking a growth response. Starch grains in certain cells of virtually all receptor organs studied respond in this way by sinking to the lower side of the cell[3]. How such lateral migration of starch grains could cause rapid changes in hormone distribution has already been discussed elsewhere[4].

The fact that organs respond to laterally applied electrostatic potential gradients has been regarded as very strong support for the geoelectric theory, although recent findings[2] call for a major revision of ideas on this phenomenon. No similar kind of support has been forthcoming for the starch-grain statoliths because so far no forces other than those arising from the mass of the grain itself (that is, gravity, centrifugal force) have been employed to shift them in the cell. In consideration of the possibilities of circumventing this difficulty, attention was given to the behaviour of organic substances in strong magnetic fields. They are normally weakly diamagnetic, and if therefore particles of such materials are placed in a *non-uniform* magnetic field, then a force will be exerted on such particles in the direction of the maximum gradient and equal to:

$$(K_1 - K_2)v.H.\frac{dH}{dx}$$

where K_1 and K_2 are the volume susceptibilities of the particle and suspending medium respectively, v is the volume of the particle, H the magnetic field strength and dH/dx the field gradient.

Few, if any, experimental observations have been made on the magnetic susceptibilities of starch or protein solution, like that surrounding statolith starch in the cytoplasm of the plant cell. Since exact experimental determination of these unknown parameters promised to involve much time and the employment of special techniques, it was resolved, in the first place, to study the behaviour of a suitable organ in a steep magnetic gradient. To this end a large permanent magnet, designed for use with a magnetron, was employed. This was fitted with soft iron pole pieces of triangular cross section as in Fig. 1. Direct measurements of the field strength by the Quincke method showed that such an arrangement gives maximum field strength just inside the narrowest point of the gap and the steepest gradient in an outward direction in the plane *A–B*, as is shown by the graph of Fig. 1. The field strength in this plane was 4,000 gauss and the gradient—5,600 gauss per cm.

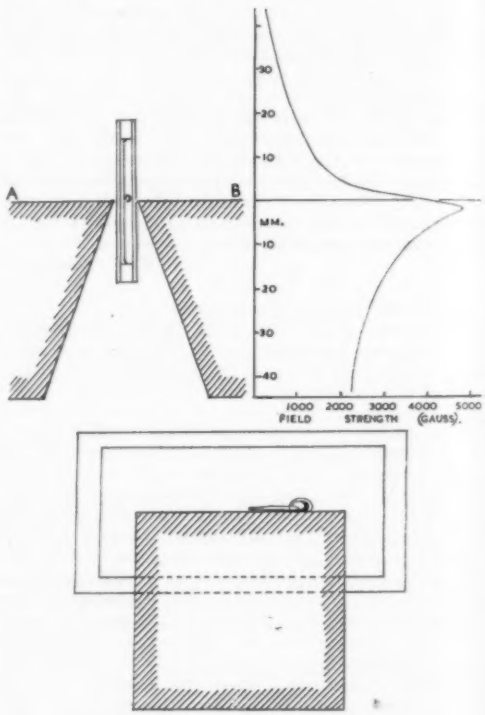

Fig. 1. Diagrams showing magnet gap design, position of glass growth chamber and cress seedling roots and field-strength distribution through the gap. Top, cross-section and field-strength curve; bottom, end view

The organ chosen for study was the seedling root of *Lepidium sativum* growing under sterile conditions on plain 2 per cent agar in a small glass chamber of design shown in Fig. 1. The seedlings were germ-

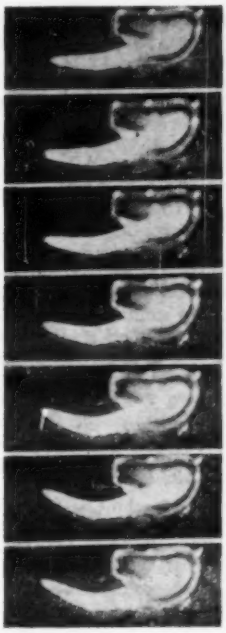

Fig. 2. Progress of curvature of 36-hr. roots of *Lepidium sativum* seedlings down the magnetic gradient

inated and grown for 36–60 hr. in the dark in an incubator at 25° C. and the roots allowed to grow downwards along the vertical surface of the agar. At the commencement of the treatment the chamber was inserted as illustrated between the poles of the magnet so that a root lay parallel to the gap and slightly above it, as near as possible to the region of maximum force. The magnet, which was held in a heavy brass cradle and mounted on an axle in ball bearings was rotated about the axis of the gap at a speed of about 2 revolutions per min. to eliminate gravitational effects which might swamp any magnetic effects. At intervals of 10–15 min. the root tip and edge of the nearest pole piece were photographed from the direction $A \rightarrow B$ by an automatic 35 mm. Robot camera provided with an 8-in. lens giving an image almost natural size on the film. The photographs were taken with synchronized electronic flash illumination of 1 μsec. duration without stopping the rotation. The direction of flash illumination was thus at right angles to the magnetic gradient; but, in any event, as shown by experiments employing visual observation only, this flash illumination had no detectable effects either on control roots out of the field or on those situated just above the gap. All these observations were made at room temperatures (18–22° C.) and in weak diffuse light.

A large number of experiments have now been performed, and in all but a very few cases roots have shown marked growth curvatures away from the gap, that is, down the magnetic gradient. Typical results for a 36-hr.-old root and 60-hr. roots are shown in Figs. 2 and 3 respectively. From the photographic records, preliminary estimates have been made of the reaction time by extrapolating backwards the curvature progress curve to cut the axis of zero curvature. In the fluctuating summer temperatures of the laboratory (18–22° C.) the values obtained fell between 30 and 45 min. Under similar environmental conditions measurements have shown the time of reaction to a continuously applied gravitation stimulus to be 4–8 min.

Having obtained such success with growth responses, attention was then focused on starch grains. First, the value of their diamagnetic susceptibilities was considered. By use of the Pascal constants it is possible to calculate from their molecular structures the magnetic susceptibilities of organic molecules. Assuming for these purposes starch grains to be amorphous the average volume susceptibility (K_{ST}) can be estimated to fall between about $-0{\cdot}74$ and $-0{\cdot}62 \times 10^{-6}$ c.g.s. units depending on the value of the density of the starch grain taken to derive it from the specific susceptibility χ. The average value of volume susceptibility for the surrounding cytoplasm (K_P) is much more difficult to assess but should differ very little from that of water, that is, $-0{\cdot}72 \times 10^{-6}$ c.g.s. units. The difference in the two susceptibilities will therefore fall between $+0{\cdot}10 \times 10^{-6}$ and $-0{\cdot}02 \times 10^{-6}$ and the resultant force on the grain may be either inwards towards the gap or outwards away from it, depending on the particular value of K_{ST}. However, starch grains are not amorphous and it is therefore possible, as has been shown for cellulose[5], that they may show appreciable diamagnetic anisotropy. If we make the reasonable assumption that the maximum value of K_{ST} along a particular axis of the grain could rise to 25 per cent above the average susceptibility calculated above, then this maximum value of K_{ST} falls between $-0{\cdot}93$ and $-0{\cdot}78 \times 10^{-6}$ c.g.s. Since the starch grain would orientate itself in the field so that its axis of maximum susceptibility would be perpendicular to the lines of magnetic flux, that is, along the maximum field gradient, then the effective force on the grain would consistently be away from the gap. It is a simple matter to get an estimate of this magnetic force in terms of the gravitational

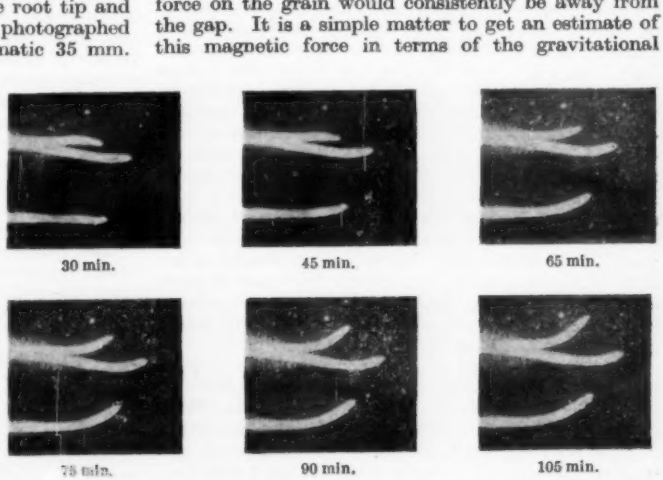

Fig. 3. Progress of curvature of 60-hr. roots of *Lepidium sativum* seedlings down the magnetic gradient

force acting on the same grain. Thus the gravitational force is expressed by the formula:

$$(\rho_{ST} - \rho_P)v.g$$

where ρ_{ST} and ρ_P are the densities of starch and protoplasm respectively. The ratio of the magnetic force to this force is given by the formula:

$$\frac{(K_{ST} - K_P)}{(\rho_{ST} - \rho_P)} \cdot \frac{H}{g} \cdot \frac{dH}{dx}$$

The value of ρ_{ST} can be estimated only approximately. Dry potato starch has a density of 1·5, which falls to 1·25 when the grain is fully imbibed. Egg white, taken as near model of cell protoplasm, gives an estimate of $\rho_P = 1{\cdot}04$. Depending then on the density taken for the statolith starch grain we obtain numerical values for this ratio ranging between 0·01 and 0·0065. This ratio is very small compared with the ratio of reaction times for magnetotropic and geotropic curvatures respectively. It is possible, however, that the ratios of presentation times, which would presumably be directly related to starch grain movement, might more nearly approach this calculated ratio of available forces.

With these possibilities in mind, attempts have been made to observe the behaviour of starch grains in roots bending in this magnetic field. Thus, with the minimum of delay after rotation was stopped, curved roots were removed from the experimental chamber and fixed in formalin–acetic–alcohol. They were first placed on the surface of a small disk of radish tissue in a film of sodium alginate dissolved in diluted indian ink, and carefully and quickly orientated thereon so that later the plane of cutting in the paraffin block could be easily and accurately identified. The whole was flooded with fixative containing 1 per cent calcium chloride simultaneously to fix the root and to turn the sodium alginate solution into a rigid calcium alginate gel. After embedding in paraffin wax, longitudinal sections were cut at 8μ in the plane of bending and stained for 24–48 hr. in dilute aqueous iodine. Measurements of starch grain distribution were made on all the root-cap cells appearing to be complete in one or more median or near median sections of well-curved roots. By using an ocular with a median hair line, which was adjusted to bisect the cell as nearly as could be judged by eye in a direction parallel to the main root axis at the tip, the numbers of starch grains on the two sides of each cell were counted and differences determined. Results of these preliminary observations are shown in Table 1. It will be seen that in most sections there is a very slight preponderance of grains in that half of the cell nearer the concave side of the root, that is, the side away from the magnet. In two sections only is this shift significant at the 5 per cent level and in most cases it is not significant. However, the overall picture is undoubtedly one of a slight shift of starch grains down the magnetic field gradient.

Suggestive as they are, these preliminary experiments do not yet constitute proof of the statolith starch theory of graviperception. They must be followed by more precise studies on starch grain behaviour in root caps and with completely unambiguous and objective methods in order to eliminate any possibility that the very small shifts recorded are not artefacts of technique or observation. In addition, exact experimental determination will have to be made of the magnetic properties of these starch grains both inside and outside the cell and of the surrounding protoplasm if suitable methods can be found for studying them. The present experiments do most strongly suggest, however, the involvement of some moving material which distributes itself in the cell under a magnetic gradient in the same sense as it does under gravity. Thus if it were a cytoplasmic particle it would have to be either denser than cytoplasm with a higher diamagnetic susceptibility, or less dense with a smaller susceptibility. However, we must not lose sight of the fact that the root as a whole will be subjected to a small outwardly directed force which will set up small bending stresses in the growing zone. The part such stresses may play in the growth responses observed must also be fully elucidated.

Table 1

Root	Section (numbered in series)	Average number of starch grains per cell	Average difference in number of starch grains. Concave–convex side	$\sigma/\sqrt{N}$	N	P
1	Median	7·40	0·79	0·37	33	0·05
2	Median	5·75	0·58	0·48	12	n.s.
3	1	5·17	0·27	0·66	11	n.s.
	2	6·1	0·5	0·63	10	n.s.
	3	6·95	1·50	0·62	20	0·03
	4	6·53	0·75	0·39	28	n.s.
	Median	6·55	0·27	0·36	29	n.s.
	Median	5·23	0·32	0·36	38	n.s.
	7	6·08	0·35	0·35	37	n.s.
	8	7·04	1·0	0·56	22	n.s.
	9	6·46	0·36	0·35	28	n.s.
	10	9·00	1·29	0·54	21	0·03
	11	8·27	−0·61	0·48	18	n.s.
	12	9·21	0·21	0·98	14	n.s.
4	1	7·43	−0·48	0·47	23	n.s.
	2	7·64	0·91	0·81	11	n.s.
	Median	8·17	0·0	0·53	24	n.s.
	3	11·01	0·17	0·73	12	n.s.
5	1	7·92	1·32	0·62	28	0·05
	2	6·00	0·3	0·36	20	n.s.
	Median	7·55	0·64	0·51	22	n.s.
	4	7·20	0·09	0·80	11	n.s.

So far the phenomenon has been investigated with only one organ of one plant species. It must be extended to roots of other species and also to other organs, such as the coleoptile, to see how universal is the correlation between the responses to gravity and those to a magnetic gradient.

Such experiments are being actively pursued, and it is hoped that this new phenomenon may prove to be a valuable tool in our attack on the problem of the mechanism of graviperception.

I wish to acknowledge my indebtedness to Dr. V. I. Little of the Physics Department, Bedford College, for many valuable discussions, for advice in the design of apparatus and for arranging the loan of the magnet used; to Dr. V. C. G. Trew of the Chemistry Department, for information on magnetochemistry and the calculation of diamagnetic susceptibility of starch, and to Dr. B. C. Sharman of the Botany Department, Bedford College, for suggesting the use of calcium alginate as a cement in the orientation of roots for embedding.

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RADIATION CARCINOGENESIS: A NEW HYPOTHESIS

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THERE is growing scientific and social concern about the possible influence of small doses of ionizing radiation upon the incidence of leukæmia and bone cancer in human beings. That large doses of X-rays (of the order of 1,000 rems) can occasionally induce leukæmia, and that long-standing radium burdens (of the order of several μgm.) in the human skeleton can occasionally produce bone cancer, are facts which are not in dispute. However, at doses and dose-rates comparable to background (generally about 0·1 rem/yr.), changes in cancer incidence are likely to escape detection because on any plausible theory they will represent only a small fraction of the normal incidence. To predict the somatic effects of small amounts of radiation we are therefore compelled to use statistics relating to high doses, and to extrapolate to low by means of a theoretical dose–response relationship.

The linear theory of carcinogenesis, which states that the incidence of radiogenic cancer is directly proportional to the radiation dose, has recently been criticized so effectively by Brues[1] that it must be regarded as being of very doubtful validity. Kimball[2] challenges the reliability of the statistics and statistical tests used by Lewis[3] in support of a linear theory of radiation leukæmogenesis. Mewissen[4], analysing the dose–response relationship in radiation leukæmia by the method of marking probits, suggests that leukæmogenesis may require more than one critical molecular event, although a single event may be the initiating agent.

Experiments in which osteogenic sarcomas in mice were induced by bone-seeking radioactive isotopes are regarded by Finkel[5] as giving evidence for a threshold effect. The low doses received by human beings from world-wide radioactive fall-out will not, she concludes, induce a single osteogenic sarcoma. By contrast, a maximum upper limit is predicted[6] from the linear theory of nearly ten cases per annum per million of population (for strontium-90 at an equilibrium-level in bone, assuming continuation of bomb tests). In view of the great diversity in these estimates, it is necessary to re-assess the evidence and to consider whether there is any alternative to the linear and threshold theories.

In this article, human radiogenic leukæmia and bone cancer will be considered, and arguments will be advanced in support of the tentative view that both diseases are initiated by a highly specific 'two-hit' mechanism.

Dose–Response Relationship in Human Leukæmogenesis

Court Brown and Doll[7] examined the statistics for the incidence of leukæmia in patients irradiated for ankylosing spondylitis and adopted the working hypothesis that there is no threshold dose, and that at low doses the incidence of radiogenic leukæmia is proportional to dose. Their results are re-plotted on log–log scales in Figs. 1 and 2. In Fig. 1, where the standardized incidence in excess of normal (that is, the radiogenic incidence) is plotted against the mean dose to the spinal marrow, it appears that a line of slope 2 gives at least as good a fit to the points as a line of slope 1. This implies that the radiogenic leukæmia incidence-rate might equally well be proportional to the square of the mean dose to the spinal marrow as to the first power. Court Brown and Doll refer, however, to the unreliability of the point at highest dose where extra-spinal marrow received a larger proportion of the dose than at the lower dose-ranges.

In Fig. 2, the radiogenic incidence-rate is plotted against the whole-body integral dose. Since the statistical errors will be similar to those in Fig. 1, the fit of the points to a line of slope 2 is very good, and it suggests that the radiogenic incidence-rate might be proportional to the square of the whole-body integral dose. These quadratic relationships have been briefly noted previously[1,8], and they hint at the possibility that radiogenic leukæmia may require a two-hit process for its initiation. It will be seen later, however, that if the hypothesis elaborated here is true, this evidence will require very extensive re-interpretation before valid conclusions can be drawn from it concerning the dose–incidence relationship.

Consideration of other evidence of human radiogenic leukæmia will be deferred until after the mathematical relationships have been presented.

Dose–Response Relationship in Human Bone Cancer

The scanty data[9] for mortality from bone cancer as a function of terminal radium burden are plotted on log–log scales in Fig. 3. They imply that radiogenic incidence is proportional to the square of the terminal radium burden. Differences in radium uptake will give rise to different deposition patterns in bone. Despite this complication, for long-term burdens there may be a rough correspondence between the square of the average terminal burden for a group and the effective mean square dose to relevant bone tissue. If this can be assumed, then for radiogenic bone cancer, as for leukæmia, there is a suggestion that a two-hit process is involved.

The formal properties of two contrasting two-hit models will now be considered.

Fisher's Multiple Mutation Theory

The idea that natural or spontaneous cancers may arise through a succession of somatic mutations has been put forward on many occasions[10], and most recently by Fisher[11], who postulates the following sequence of events: (1) a somatic mutation; (2) clone growth from the mutated cell; (3) a second mutation within a clone cell which, in the case of a

two-mutation sequence, will give rise to cancerous growth.

In the case of a three-mutation sequence, (3) above is followed by: (4), a second clone-growth of cells containing two mutations, and (5), a third mutation within this clone leading to tumour development.

Fisher also argues that in epithelial tissues it is likely that the number of mutated cells in a clone increases (after the first few divisions) in proportion to the square of the time. If it is considered that a *natural* cancer arises as a result of n successive spontaneous mutations, and if mutation-rates and clone growth-rates are invariant with respect to age, the age-specific incidence-rate is shown by Fisher to be proportional to $(\text{age})^{2n-1}$. Thus, for $n = 2$, the age-specific incidence-rate would be proportional to $(\text{age})^3$ and for $n = 3$ it would be proportional to $(\text{age})^5$. Court Brown and Doll[12] have examined the natural death-rate versus age relationships for chronic lymphatic, chronic myeloid and acute leukæmias. With chronic lymphatic leukæmia there is probably no genuine cohort effect[13] (that is to say, the actual death-rate from chronic lymphatic leukæmia at a given age is not changing with time) and the age-specific death-rate is proportional to $(\text{age})^6$; thus, assuming the difference between age at incidence and age at death will not appreciably distort the relationship, a triple-mutation sequence would be implied. For chronic myeloid leukæmia it is again likely that there is no genuine cohort effect[13], and for this disease the age-specific death-rate is proportional to $(\text{age})^3$, suggesting a double-mutation sequence. Acute leukæmia shows a marked cohort effect between the years 1945 and 1957 and the shape of the age-specific death-rate versus age curve is unusual, with mortality falling from adolescence to a minimum at 25–29 years, and then increasing slowly up to 70 years. Since the cohort effect suggests the presence of an extrinsic leukæmogenic agent, no reliable conclusion can be drawn about a possible intrinsic death-rate versus age relationship, except that from Court Brown and Doll's graph it appears unlikely to be of the sixth-power type.

It may well be significant that radiation exposure has been associated with chronic myeloid and acute leukæmias, but not with chronic lymphatic leukæmia requiring (hypothetically) a three-hit rather than a two-hit sequence.

Equally important is the connexion between age-specific death-rates from bone cancer and age. It is possible that there is no genuine cohort effect where incidence (as opposed to death) is concerned[13], and plotting from Case[13] the mean age-specific death-rate from cancer of bones (excluding jaw) for the 1866 and 1871 cohorts against age on log–log scales, we find, from Fig. 4, dependence on $(\text{age})^3$.

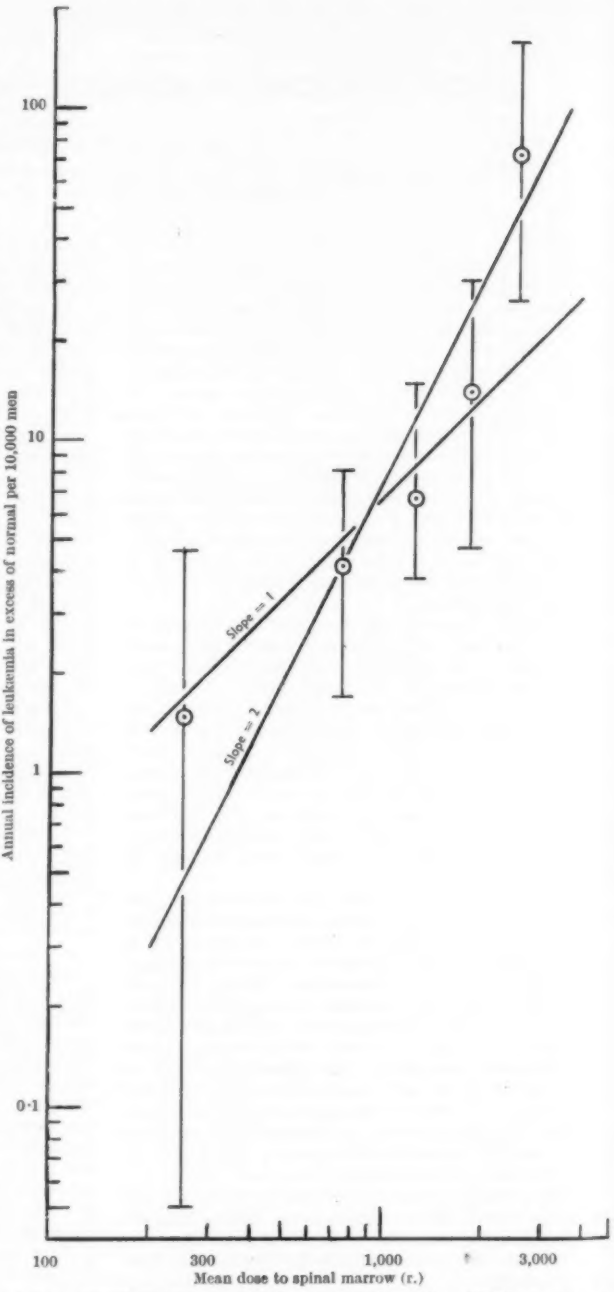

Fig. 1. Annual incidence of leukæmia in excess of normal per 10,000 men, versus mean dose (r.) to spinal marrow. Re-plotted from Court Brown and Doll (ref. 7) on log–log scales. Errors are taken from the original paper and represent 95 per cent confidence limits based simply upon the number of cases. The actual errors will be slightly larger

Further investigation will, however, be necessary to ascertain whether the classification 'bone tumours excluding jaw' refers predominantly to the same type of tumour as that induced by radium in bone. Nevertheless, for bone cancer, as for chronic myeloid

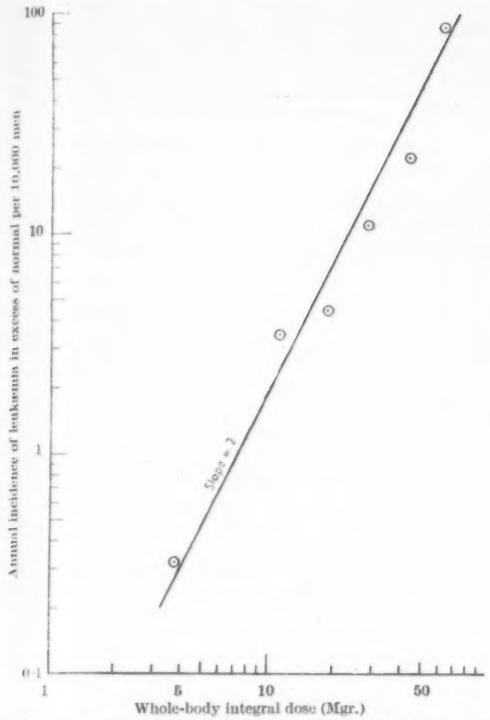

Fig. 2. Annual incidence of leukæmia in excess of normal per 10,000 men versus whole-body integral dose (Mgr.). Re-plotted from Court Brown and Doll (ref. 7) on log–log scales. The errors were not calculated, but they will be similar to, or slightly larger than, those in Fig. 1

leukæmia, it is possible that the natural incidence requires a two-mutation sequence and that radiogenic incidence requires a two-hit process.

Consider now the application of Fisher's theory to radiogenic cancer. Suppose the radiation dose is delivered over a long period (as with background or fall-out) and at a constant rate R rems per unit time.

Suppose m_1 = number of specific first mutations produced per rem, m_2 = number of specific second mutations per rem.

α = growth-rate constant for clones containing 'first-mutation cells'. (Fisher assumes for epithelial tissues that the number of cells in a clone increases in proportion to the square of the time, and we shall adopt the same growth law here.)

It can be shown that dN_2, the number of cells arising containing both specific mutations during a time interval t to $t + dt$ is given by :

$$dN_2 = \tfrac{1}{3}\,\alpha m_1 m_2\, R^2 t^3\, dt \qquad (1)$$

Since the total dose delivered is $D = Rt$,

$$dN_2/dt = \tfrac{1}{3}\,\alpha m_1 m_2\, D^2 t \qquad (2)$$

Thus, on this theory, if a two-mutation sequence is required for carcinogenesis, the rate of radiogenic tumour cell formation will be proportional not only to the square of the dose but also to the time over which it is delivered (provided t is large compared with the time of first-mutation cell division). In the same manner, widely spaced (that is, fractionated) doses should be extremely effective as compared with the same total dose delivered in a short interval.

We shall see later that, in spite of large uncertainties in the evidence, there is no indication that low dose-rates or fractionated doses possess this enhanced efficiency. Indeed, it is much more likely that low dose-rates are, in general, less effective than high.

Fisher's multiple mutation theory seems inapplicable, therefore, to radiation leukæmogenesis in humans, although where there is no cohort effect it offers an attractive interpretation of the dependence of the natural age-specific incidence-rate on age.

Summarizing, certain natural cancers may arise as a result of two or three successive somatic mutations, but radiogenic leukæmia and bone cancer may well originate from two discrete events which have certain properties in common with, but others different from, natural somatic mutations. One is, therefore, led to the conjecture that these discrete events in the radiation case could be chromosome or chromatid breaks. At the same time, it is necessary to assume that, after the first break, clone-growth does not occur, although in the eventual cancer cell the 'break' (gene deletion ?) must produce an effect similar to that of the natural mutation.

Chromosome-break Model

In this model it is assumed that a radiogenic cancer arises from a cell which has received two

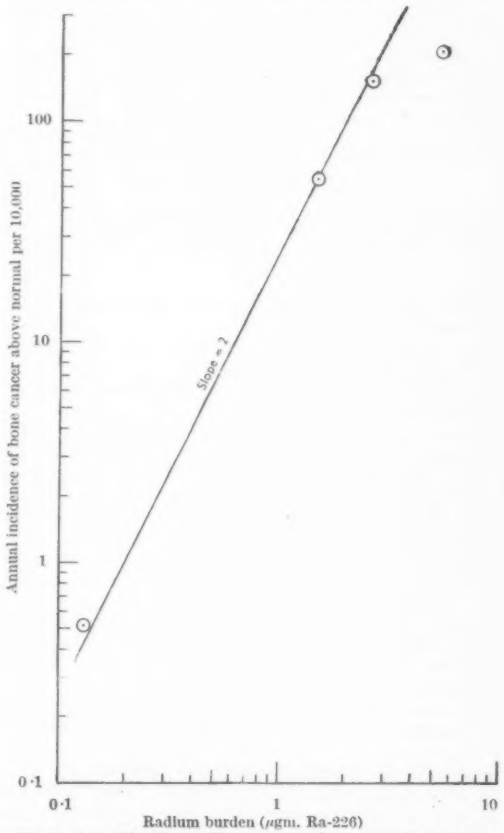

Fig. 3. Annual incidence of bone cancer above normal per 10,000 versus terminal radium burden (μgm. radium-226). Re-plotted on log–log scales from Marinelli (ref. 9). No error estimates were given in the original paper

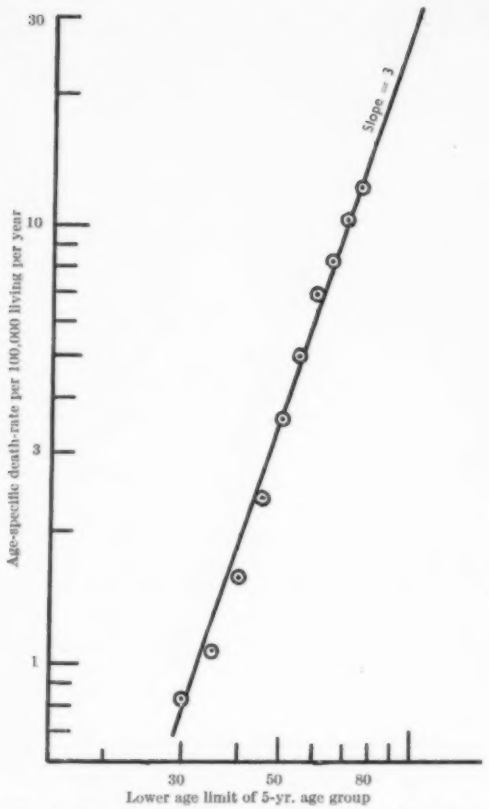

Fig. 4. Mean age-specific death-rate from cancer of bones (excluding jaw) for the 1866 and 1871 cohorts, versus age, on log-log scales. Plotted from Case's (ref. 13) analysis of statistics for England and Wales

specific chromosome breaks. The following detailed suppositions are made :

(*a*) L cells of a particular tissue type are capable of developing into cancer cells.

(*b*) They are irradiated at a constant dose-rate of R rems per unit time.

(*c*) c_1 is the average number of specific first chromosome breaks produced per cell per rem.

(*d*) Cells containing a 'c_1' break may be rendered carcinogenically ineffective through natural processes (such as repair or cell death), or through further radiation injury (such as another chromosome break) leading, perhaps, to cell death. The natural processes of elimination are characterized by a simple exponential function with decay constant λ (though the function could well be multiple exponential, Gaussian, or of some other form). p, the number of lethal radiation hits per 'c_1–type cell' per rem characterizes the radiation injury.

(*e*) c_2 is the average number of specific second chromosome breaks produced in a 'c_1–type' cell per rem.

(*f*) A long interval can elapse between the production of a potentially cancerous cell and the actual development of a cancer. If during this period, or a part of it, cancerous potentialities are carried by only one cell, the receipt of a further radiation 'hit' (producing, for example, another chromosome break) could be lethal to that cell. q is the number of such lethal hits per potentially cancerous cell per rem.

If the irradiation time is short so that a potentially cancerous cell cannot undergo division during the irradiation, it can be shown that the number, N_2, of potentially cancerous cells is given by :

$$N_2 = \frac{Lc_1c_2R^2}{\lambda + pR}\left\{\frac{1 - e^{-qRt}}{qR} + \frac{e^{-(\lambda + pR)t} - e^{-qRt}}{\lambda + R(p - q)}\right\} \quad (3)$$

(In solving the differential equations imposed by the assumptions, it is further assumed that at the commencement of the irradiation there are no c_1- or c_2-type breaks in any of the L cells.)

(*g*) If a potentially cancerous cell divides shortly after its production and cancerous potentialities are transmitted to both daughter cells, the chance of both cells being eliminated by subsequent radiation damage is very much reduced. If the 'vulnerable period' (τ) between the initial production of the potentially cancerous cell and its effective multiplication is short (such that $qR\tau \ll 1$), q-type lethal hits can be disregarded. For protracted irradiations under these conditions we obtain :

$$N_2 = \frac{Lc_1c_2R^2}{\lambda + pR}\left\{t - \frac{1 - e^{-(\lambda + pR)t}}{\lambda + pR}\right\} \quad (4)$$

The significance of equations (3) and (4) is best understood with reference to various limiting conditions. Consider first the dose–response relationship for small doses delivered at a high dose-rate, so that the following conditions obtain :

$$pR, qR \gg \lambda$$

$$\lambda t, pRt, qRt \text{ (that is, } pD \text{ and } qD) \ll 1$$

Equation (3) will be applicable and it reduces to :

$$N_2 = Lc_1c_2D^2/2 \quad (5)$$

If, however, a small dose is delivered at a low dose-rate over a very long period (as with background and fall-out radiations) such that $\lambda t \gg 1$, $\lambda \gg pR$,

$$pD, qD \ll 1$$

equation (4) reduces to :

$$N_2 = Lc_1c_2R^2t/\lambda = Lc_1c_2D^2/\lambda t \quad (6)$$

Under these conditions, cancer incidence will be less than that observed for the same dose delivered at high dose-rate in the ratio of $2/\lambda t$ ($\lambda t \gg 1$).

Consider now the effect of a large dose delivered at a high dose-rate in a short time,

$$\text{that is, } pD, qD \ll 1$$
$$pR \gg \lambda$$

Equation (3) is applicable, and it has been shown above for small doses at a high dose-rate that N_2 is proportional to D^2. As pD and qD increase with increasing dose, they will become an appreciable fraction of unity, and N_2 will fall below proportionality to D^2. In the limiting case where pD, $qD \gg 1$, N_2 reaches a plateau :

$$N_2 \to \frac{Lc_1c_2}{pq} \quad (7)$$

The remaining case to consider is the delivery of a large dose over a long period.

$$t \gg 1, \lambda t \gg 1, pD \gg 1$$

Equation (4) applies and it simplifies to :

$$N_2 = \frac{Lc_1c_2R^2t}{\lambda + pR} = \frac{Lc_1c_2D^2}{\lambda t + pD} \quad (8)$$

Before proceeding to a rough estimate of the constants in these equations, and in order to relate N_2 (total incidence) to observed incidence-rates, it is necessary to consider some of the possible biological processes involved in carcinogenesis.

Carcinogenesis : Possible Mechanisms

We have seen that on Fisher's multiple mutation theory the dependence of the natural age-specific incidence-rate on age for chronic myeloid leukæmia and for bone cancers can be understood if two successive somatic mutations are required within the same cell. It is also possible that radiogenic leukæmia and bone cancers result from two chromosome or chromatid breaks within the same cell : these breaks could produce gene deletion. It is tempting to conclude that the two mutations and the two breaks have a similar effect on the economy of the cell, and that in both cases the modified deoxyribonucleic acid will produce a modified ribonucleic acid synthesis. Modified ribonucleic acid will in turn affect protein synthesis in the cytoplasm.

On the basis of immunological studies, Green[14] contends that malignancy arises through the following stages : initially there is a modification of one or more cytoplasmic specific protein complexes (cell identity markers) ; these modified complexes then elicit an antibody response, to which the cell eventually 'adapts' by losing the modified marker. This loss of marker involves the failure of the normal growth-regulating mechanism and the 'adapted' cell becomes neoplastic. Other evidence points to a rather similar conclusion, that certain proteins essential to normal controlled division are either eliminated (corresponding to Green's antigenic loss) or structurally modified so as to be incapable of functioning normally[15].

Modified protein synthesis could result in irreversible injury of respiration which, Warburg[16] has claimed, is the essential first phase of cancer. The present hypothesis does not, however, predict such a specific form of injury. Thus, although there is no contradiction with Warburg's theory of cancer, the present hypothesis is, in its most important aspects, independent of it.

Osgood[17] argues that a basic dividing cell must generally give rise to one cell which proceeds to differentiate and another basic or primitive cell which is capable of maintaining this process through further division. This normal or 'arithmetic' type of division will occasionally (during growth or after injury) change to 'logarithmic' division in which two basic daughter cells emerge after cell division. Osgood postulates that logarithmic division is normally prevented by an inhibitor which, he suggests, is produced in the adjacent or nearly adjacent differentiated cells. Malignancy is said to result from a lowered concentration of inhibitor, and Osgood claims that a reduction in the life-span of differentiating cells could bring this about.

Although some such homœostatic control must exist, logarithmic growth could arise not only via Osgood's suggested mechanism but also through any appropriate interruption in the feed-back circuit and in particular through the malfunctioning of the 'inhibitor receptors' in the basic dividing cells. (These 'inhibitor receptors' may be equated with Green's cell identity markers.) If a unique two-hit process is involved in radiogenic leukæmia and bone cancer, it would seem that the initiating process is much more likely to involve a modification of the 'inhibitor receptors' than injury to one, or at the most two, differentiated cells. Osgood's explanation in terms of a reduced inhibitor concentration could, of course, apply in certain other situations where, for example, hormonal factors are important.

The biological mechanism of 'two-hit' radiation carcinogenesis favoured here is therefore as follows:

When a viable cell containing a c_1-type chromosome modification receives a c_2-type chromosome (chromatid) break, the resulting cell is potentially carcinogenic. Subsequent cell division will dilute the normal ribonucleic acid and the normal cell identity markers initially present until eventually a cell emerges in which normal markers have been entirely replaced by modified markers. At this stage (or possibly before) the antibody response postulated by Green will be elicited. Adaptation, involving the elimination of all markers, produces the carcinogenic cell.

The number of cell divisions required to attain the necessary dilution of cell markers will be affected by chance. Hence, an irradiation of a group of similar organisms should be followed by a minimum latent period, at the end of which the tumour incidence-rate should show an approximately normal or Poisson distribution in time.

The Hiroshima statistics[18] suggest that this may be true in the case of radiogenic human leukæmia, with peak incidence-rate occurring about seven years after irradiation (Fig. 5). On this hypothesis, the minimum latent period should also be independent of dose, except that heavy doses, by extensive cell destruction, could accelerate cell division among potentially malignant cells and produce a shortening of the minimum latent period. This relative independence of latent period on dose is also supported by the Hiroshima leukæmia statistics.

If the dose-range is low enough so that incidence (N_2) can be expected to be proportional to D^2 (that is, pD, $qD \ll 1$), then beyond the minimum latent period, cumulative tumour incidence and incidence-rate at a given time after the commencement of irradiation should both be proportional to the square of the dose.

Brues's experiments[19], in which the daily probability of bone tumour appearance in mice was observed as a function of strontium-89 dose, support this contention, although previously they have been differently interpreted. Where there is a time overlap between incidence-rate curves at different doses, the probability of incidence at a given time is, within experimental error, approximately proportional to $(\text{dose})^2$. This is true for both continuous and single injection experiments. The apparent lengthening of the latent period at the lower doses is therefore due, in part at least, to inadequate statistics, for when doses differ by a factor of 10, incidence will differ by a factor of 100 ; consequently, significant increases above the normal or spontaneous incidence emerge much sooner at high doses.

Finkel and Biskis[20], also on the evidence of experiments with mice, conclude that the latent period for the induction of osteogenic sarcomas is independent both of isotope and of dose. If this deduction is true, then according to the present hypothesis two consequences should follow. First, in their Fig. 4, where the slope of the changing tumour-rate is plotted against injected dose (on log–log scales), the curves should have a slope of 2 over the lower dose-ranges. (This follows if N_2 is proportional to the square of the dose and if tumour-rate is distributed in time

according to an error function the width of which is independent of dose.) For strontium-90 the slope is slightly less than 2, but for calcium-45 it is slightly more than 2; the data for radium-226 are too few to allow the (steep) slope at low dose to be determined, but at high dose the slope becomes less than unity, implying, as would be expected, that p and q for α-particles are somewhat greater than for β-particles. Secondly, the cumulative incidence of osteogenic sarcomas before death intervenes should at a given time (and at low dose) be proportional to the square of the dose. Judging from Finkel and Biskis's Fig. 1, this relationship, in general, does not hold, although the discrepancy could be explained if, contrary to their deductions, the latent period at high dose is, in fact, reduced.

In connexion with these experiments on mice, it should be mentioned that Marinelli[9] has estimated that at low doses the relative biological effectiveness of α-particles is 4. It is interesting to inquire what combination of radiation damage to the chromosome material could yield this relative biological effectiveness. Some important data concerning the frequency of chromosome and chromatid breaks are assembled in Lea's[21] Table 67, and although they refer to *Tradescantia* microspores they will be used here as a guide. If a two-hit mechanism in the organism consisted of one chromatid break which joined and one which remained unjoinable, the relative biological effectiveness of α-particles (relative to X-rays) can be deduced from Lea's table to be approximately 4.

If radiogenic bone tumours in mice were produced by somatic mutations rather than through breaks in the chromosome material, we should expect the relative biological effectiveness of α-particles to be less than unity. The relative biological effectiveness evidence for radiogenic bone tumours in mice is therefore consistent with the chromosome (chromatid) break hypothesis and at variance with the multiple-mutation theory.

It is not intended to suggest that the present 'two-hit' or 'double-break' hypothesis is applicable to all cases of radiation carcinogenesis; thus in mice, radiogenic thymic leukæmia and ovarian neoplasia may very well be produced by a 'threshold' or generalized-damage mechanism. I hope to discuss the 'threshold' concept on another occasion.

Application of Chromosome-break Hypothesis to Human Leukæmogenesis

The numerical results derived in this section must be treated with the utmost reserve since uncertain data are substituted in an extremely tentative hypothesis. It may be said, however, that the same is true of all the previous estimates of radiogenic leukæmia incidence.

The Hiroshima and Nagasaki leukæmia statistics would be of immense theoretical importance if it were possible to estimate accurately the dose to the survivors. In the absence of detailed information, an attempt has been made to obtain very approximate averages for the Hiroshima survivors using the following procedure. Air doses for γ-rays and fast neutrons were taken from curves prepared by the Health Physics Division of Oak Ridge National Laboratory[22]. The mean attenuation of γ-rays is assumed to be 0·76 in buildings and 0·74 in tissue overlying bone marrow. The mean attenuation of fast neutrons is assumed to be 0·48 in buildings and 0·26 in tissue overlying bone marrow; the relative

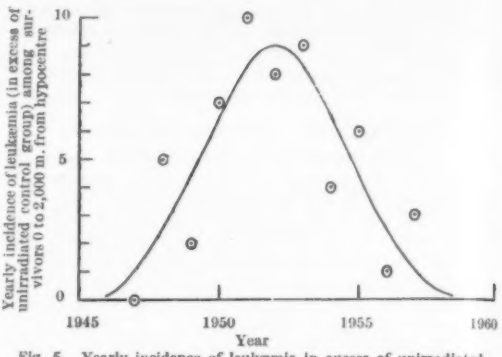

Fig. 5. Yearly incidence of leukæmia in excess of unirradiated control group, among the survivors at Hiroshima who were 0–2,000 m. from the hypocentre, versus year. Plotted from Wald (ref. 18)

biological effectiveness for fast neutrons is taken to be 2. Fatalities were very high in the inner zone (0–1,000 m. from the hypocentre) and most of the leukæmia victims were in the region 850–1,000 m.[3]. Here, the average bone marrow dose (assuming a uniform geographical distribution of the population) is found by integration to be 600 rems. Similarly for the zone 1,000–1,500 m., the average is 170 rems. For the zone 1,500–2,000 m., where incidence is expected to be proportional to (dose)[2], the mean square dose has been calculated and found to be nearly 700 (rems)[2]; the root mean square dose is, therefore, 26 rems. The mean or root mean square doses are plotted in Fig. 6 versus the mean leukæmia incidence-rate[18] for the period 1950–57 on log–log scales. The points are consistent with the type of relationship predicted by equation (3). That is, at low doses, incidence is proportional to (dose)[2], but at high dose the curve bends over and attains a plateau.

If the incidence of radiogenic leukæmia for the lowest dose group is distributed in time according to the smooth curve of Fig. 5, and if no other peaks occur in future years, the total incidence of radiogenic leukæmia for this group is calculated to be 1·5 per 10,000.

From equation (5):

$$Lc_1c_2 = \frac{2N_2}{D^2} = \frac{3{\cdot}0 \times 10^{-4}}{694}$$

$$= 4{\cdot}3 \times 10^{-7}$$

Brues[1], on the assumption that leukæmia arises from proliferating myeloid cells, has suggested that L is of the order of 10^{11} cells.

$$\text{Hence } c_1c_2 \sim 4 \times 10^{-18}$$

Assume that c_1 and c_2 refer to two chromatid breaks, one of which is restituted and the other remains broken. If a fraction 0·09 of primary breaks are unjoinable[21], then if c_1 and c_2 are now referred to primary breaks,

$$(c_1c_2)_{\text{primary breaks}} \sim \frac{4 \times 10^{-18}}{0{\cdot}09 \times 0{\cdot}91}$$

$$\sim 5 \times 10^{-17}$$

Since c_1 and c_2 are likely to be of the same order of magnitude:

$$c_1 \sim c_2 \sim 7 \times 10^{-9} \text{ specific primary breaks/rem}$$

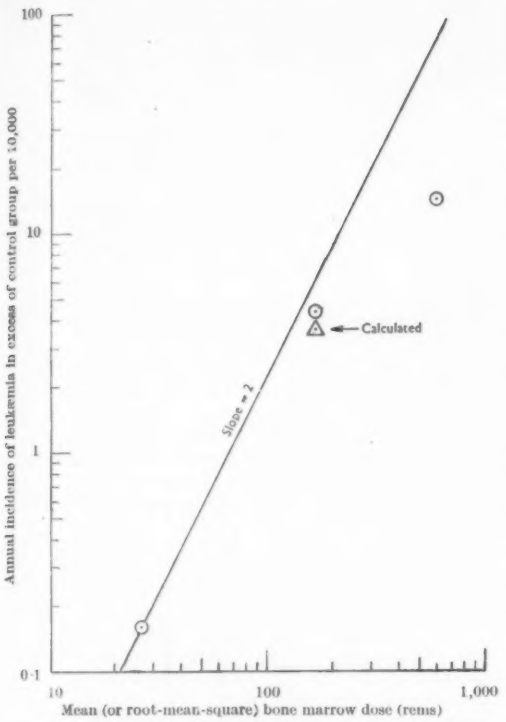

Fig. 6. Mean annual incidence of leukæmia at Hiroshima above unirradiated control group per 10,000 for 1950–57, versus mean, or root-mean-square, dose to bone marrow (log–log scales). The leukæmia statistics (circles) are taken from Wald (ref. 18) and the doses are calculated from Ritchie and Hurst (ref. 22) (see text). The point enclosed in a triangle is calculated from the present hypothesis (see text)

On the basis of Lea's target theory[21], this figure is found to correspond to a target molecular weight for each specific primary break of the order of 10^4. This reasonable value contrasts with the absurdly small target volumes entailed by the linear theory of carcinogenesis and commented on by Brues[1].

It is also apparent that if a tumour required for its initiation the co-existence of three specific chromosome (chromatid) breaks in one cell, then for any reasonable values of L, c_1, c_2 and c_3 the chance of a radiogenic tumour arising from doses of the order of 1,000 rems must be exceedingly small. Thus, if and when radiogenic tumours arise from specific modifications to the chromosome material of a single cell, only those tumours are likely to be observed which require two such modifications. (It is possible, however, to account for the occurrence of natural tumours requiring three successive mutations on the hypothesis of clone-growth of mutated cells.)

For the point at highest dose in Fig. 6 it can be shown that equation (7) is a good approximation, hence:

$$pq = \frac{Lc_1c_2}{N_2}$$

From Wald's[18] statistics and the smooth curve of Fig 5, $N_2 = 1{\cdot}39 \times 10^{-2}$ leukæmias per person. Therefore,

$$pq = \frac{4{\cdot}3 \times 10^{-7}}{1{\cdot}39 \times 10^{-2}} = 3{\cdot}1 \times 10^{-5}$$

If, as is probable, p and q are of the same order of magnitude, $p \sim q \sim 6 \times 10^{-3}$ lethal hits per cell per rem.

This figure may be compared with the values $7{\cdot}2 \times 10^{-3}$ chromatid breaks per cell per r., and 6×10^{-4} chromosome breaks per cell per r., quoted by Lea[21] for the production of aberrations in *Tradescantia* microspores by high-energy X-rays.

The expected incidence-rate for the mid-point in Fig. 6 can now be calculated from the constants derived above, if it is assumed that p is not identically equal to q. (Since R here is very large, it is highly probable that in equation (3), $R(p - q) \gg \lambda$. To achieve this it must be assumed, $p \neq q$.) Fortunately, the value of N_2 is not critically dependent on p and q, and putting $p = 6 \times 10^{-3}$, $q = 5 \times 10^{-3}$, we obtain $3{\cdot}6 \times 10^{-4}$ leukæmias per person per year (for the years 1950–57), which is in satisfactory agreement with the observed value of $4{\cdot}3 \times 10^{-4}$.

Finally, the statistics for leukæmia incidence among American radiologists enable a crude estimate of λ to be made. It must, however, be emphasized that differences (for example, in age and sex distributions) between this group and the Hiroshima survivors make the value of such a derivation somewhat dubious. Over the period 1938–52 the average death-rate among American radiologists from radiogenic leukæmia was approximately 4·9 per 10,000 per annum[3]. Deaths within the age-range 35–74 years were recorded and the average expectation of life was 62 years. Assuming equilibrium conditions, then for radiologists older than 35 years, the total expectation of radiogenic leukæmia would have been approximately $(62–35) \times 4{\cdot}9 \times 10^{-4}$, that is, $1{\cdot}32 \times 10^{-2}$. The mean total exposure time to radiation was 24·4 years[3]. Braestrup estimates that the typical exposure dose-rate to radiologists working under poor protection conditions was about 100 r. per annum, corresponding to a dose-rate to the bone marrow of 66 rems per annum[23]. With the subsequent improvement in protection, the marrow dose-rate has now fallen to less than 1 rem per annum. Assuming that equation (8) is applicable, we may take R to be (very roughly) 50 rems per annum. Solving for λ, we obtain the value $1{\cdot}_{75}$ yr.$^{-1}$, but since it depends on almost the square of the (uncertain) dose-rate, the error could, perhaps, amount to one order of magnitude. (This assumes that we are dealing with a simple exponential function—and this assumption might well be false.)

Predicted Incidence of Radiogenic Leukæmia at Low Dose-rates

It is interesting to apply this hypothesis to the incidence of radiogenic leukæmia to be expected from background and fall-out radiations.

Background radiation. The following assumptions are made:

$$R = 0{\cdot}1 \text{ rem/yr.} \quad t = 70 \text{ yr.}$$

$$\frac{Lc_1c_2}{\lambda} = \frac{4{\cdot}3 \times 10^{-7}}{1{\cdot}75} = 2{\cdot}5 \times 10^{-7}$$

(In the absence of better information this is taken to be constant throughout life, although obviously L, at least, will vary considerably.)

Applying equation (5), $N_2 \sim 1{\cdot}7 \times 10^{-7}$.

Thus, neglecting problems of latent period, in a population of 50 million people of average life-expectancy 70 years, background radiation may

Table 1

Radiation	Dose-rate assumed (rems/yr.)	Duration of exposure (yr.)	Incidence-rate of radiogenic leukæmia		
			Linear theory (after Lewis, ref. 3)	Present 'two-hit' theory. Possible range	
Natural background	0·1	70	25,000	1 to 100	per 50 million persons in 70 years (as compared with nearly 200,000 'natural' cases)
Fall-out	0·01	70	2,500	0·2 to 20	
Maximum permissible occupational exposure	5	40	32	0·1 to 10	in a population of 4,000 over a life-time of occupational exposure (as compared with 15 'natural' cases)

The effect of low dose-rate ionizing radiations on radiogenic leukæmia incidence in humans. A comparison between predictions made (*a*) on the basis of the linear theory (after Lewis, ref. 3) and (*b*) by the 'two-hit' hypothesis presented here. For reference, the 'natural' total incidence based on present statistics for England and Wales is shown in the last column.

induce of the order of ten cases of radiogenic leukæmia over a 70-yr. period.

This is exceedingly small compared with the 'natural' incidence-rate in Great Britain of about fifty cases per million persons per annum. It is also very small compared with the best predictions made with the linear theory. Thus, Lewis[3] estimates for the 'average' individual in a population that the probability of radiation-induced leukæmia is 2×10^{-6} per rem per annum. Hence, in a population of 50 millions, the total number of radiogenic leukæmias appearing over a 70-yr. period for a dose-rate to each individual of 0·1 rem per annum would be:

$$\simeq 5 \times 10^{7} \times 2 \times 10^{-6} \times 0{\cdot}1 \times \frac{70^{2}}{2}$$

$= 25{,}000$ (as compared with a possible range of 1–100 predicted above).

Fall-out radiation. A dose-rate to the individual from internal and external sources of 0·01 rem/yr. will be assumed (this is probably in excess of contemporary dose-rates from fall-out). If this is added to the normal background, and if the influence of other sources of radiation is ignored, the additional incidence, ΔN_2 of radiogenic leukæmia is given by:

$$\Delta N_2 = \frac{Lc_1c_2t}{\lambda}\,(0{\cdot}11^2 - 0{\cdot}10^2)$$

$$\sim 3{\cdot}6 \times 10^{-8}$$

(According to this hypothesis, ΔN_2 depends upon the normal background dose-rate.) Thus, in a population of 50 million people of average life-expectancy 70 years, an increase of the radiation background from 0·1 to 0·11 rem/yr. should produce of the order of two additional cases of radiogenic leukæmia over a 70-yr. period. Lewis's relationship predicts 2,500 cases under these conditions. (Being based on a linear theory, this figure is unaffected by the level of natural background radiation.)

Maximum Permissible Occupational Exposure Dose

In the United Kingdom, the maximum permissible life-time dose to the blood-forming organs of radiation workers is 200 rems. If this dose is spread uniformly over forty working years ($R = 5$ rems/yr.) we find from equation (3) that $N_2 \sim 2{\cdot}5 \times 10^{-4}$ leukæmias per person. Hence, in a population of 4,000, exposed for an occupational life-time at the maximum permissible dose-rate, we should, according to this hypothesis, expect of the order of one radiogenic leukæmia to be induced. On the basis of Lewis's[3] linear relationship, the corresponding figure would be thirty-two cases; at this higher dose-rate the disparity between the two theories is much narrower.

Osteogenic Sarcoma

The available data relating to human osteogenic sarcoma are too scanty to enable numerical predictions to be made for low dose-rate conditions with the present hypothesis.

Before concluding, it should be mentioned that alternative two-break hypotheses can be entertained. We can, for example, conceive a model in which $\lambda = 0$ and c_1 and c_2 are functions of dose-rate (R). Starting from the same statistics of human radiogenic leukæmia, the predictions from such a model would probably not differ greatly from the predictions made here. However, there is no obvious way at present of establishing a relation between c_1, c_2 and dose-rate.

Finally, any plausible alternative to the linear theory is likely to predict a very much lower incidence of radiogenic tumours at low doses and low dose-rates. It is difficult to imagine a realistic hypothesis which would lead to predictions intermediate between linear (one-hit) and quadratic (two-hit) theories, while any 'threshold' theory would be likely to involve an altogether negligible incidence at dose-rates comparable to background.

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OBITUARIES

Dr. R. A. Frazer, F.R.S.

THE news of the death of Dr. R. A. Frazer at his home in Ockham on December 10 was received with much sadness by his former colleagues at the National Physical Laboratory, and particularly by those who had had the privilege of working with him or under his leadership. His unselfishness, charm, modesty and humour made him a most likeable person and he will be sadly missed by many friends. His scientific ability and distinguished bearing inspired respect in all who met him, and his kindliness won the affection of all those who had the good fortune to know him well.

Dr. Frazer had a brilliant career as a mathematician at Cambridge and was a Rayleigh Prizeman. He joined the staff of the National Physical Laboratory in 1914, his first post being that of assistant secretary in administration. After about a year he was transferred to a group engaged in aerodynamic research in the Engineering Division. This group of pioneers in aeronautics became the nucleus around which the Aerodynamics Division was formed a few years later.

During the first ten years or so of Frazer's career as a research worker, his mathematical talent was put to good use in the study of problems of viscous flow, but from time to time he took part in other investigations. Some of the work he did in connexion with full-scale tests on the airship *R*.38 resulted in his winning the *R*.38 Memorial Prize awarded by the Royal Aeronautical Society.

It was characteristic of him to do everything he attempted with great thoroughness, and he was never satisfied until he had unravelled all aspects of any problem, however complicated, on which he happened to be engaged. He had remarkable power for concentration and when deeply immersed in a problem would lose all sense of time.

It was not surprising that, when aircraft flutter became a serious problem around about 1924, Frazer was asked to investigate what was then a mysterious phenomenon. By wind tunnel tests on simple aircraft models of reduced elasticity, and by analysis, he and his co-workers were soon able to obtain a physical understanding of flutter and to discover methods for its prevention. With W. J. Duncan as co-author he published in 1928 the first flutter monograph, and this is now often referred to as the 'flutter bible'. In this book the phenomenon is clearly explained by reference to simple systems, and the basic theory for the prediction of critical speeds for flutter is fully developed. His study of flutter theory led him to take an interest in matrix algebra for the purpose of analysing complicated systems involving many degrees of freedom. The outcome of this work was a book on "Elementary Matrices", written with Duncan and Collar as co-authors. This is now regarded as a classic on the subject, and it has had a profound world-wide influence on the development of methods of flutter and vibration analysis and on the general theory of aero-elasticity.

In recognition of his outstanding work he was elected a Fellow of the Royal Society in 1946. He was also a Fellow of the Royal Aeronautical Society and of the Institute of the Aeronautical Sciences, and was a member of the Oscillation Sub-Committee of the Aeronautical Research Council. Formerly he was also a member of the Mechanics Committee.

Immediately after the Second World War, the National Physical Laboratory was asked to investigate the possibility of wind-excited oscillations of suspension bridges, in connexion with the proposal to erect suspension bridges across the Severn and the Forth. It was feared that, like the Tacoma bridge, these bridges would fail unless they were properly designed to avoid such instability. Again it was Dr. Frazer who took responsibility for the investigation. With the assistance of C. Scruton and other members of the Division, he designed and made a 50-ft. long reduced elasticity model for testing in a specially erected wind tunnel at Thurleigh. In due course they were able to reproduce on the model the kind of oscillation that destroyed the Tacoma bridge and show how it could be avoided by suitable design. As a result of their investigations they were able to propose a design for the Severn and Forth Suspension Bridges which was not prone to instability in the strongest winds. A bridge to this design is now being built over the Firth of Forth.

Outside his work, Dr. Frazer had many interests. In his younger days he was a keen mountaineer and enjoyed nothing better than climbing in the Alps, the Lake District and North Wales. He was also a member of Oxford University Expeditions to Spitsbergen in 1921, 1923 and 1924. In later years his main interest was gardening. When he retired in 1954 after forty years service, everyone hoped that he would have good health to enjoy a long and happy retirement with his family, but this was not to be.

Dr. Frazer's name will live long in aeronautical history, and he will always be remembered with esteem and affection by many friends who feel the deepest sympathy with his wife and family in their bereavement.

W. P. JONES

Prof. Anrezej Soltan

THE death has been announced of Dr. Anrezej Soltan, director of the Polish Institute of Nuclear Research since 1955. Born in 1897, Dr. Soltan was a member of the Scientific Council, Joint Institute for Nuclear Research, Dubna, U.S.S.R., a member of the Polish Academy of Sciences, and he held the position of chairman of the Central Administration of the Polish Physical Society during 1952–54.

Soltan was educated at the University of Warsaw and carried out scientific work in France during 1927–28 and in the United States during 1932–33. His publications cover the fields of spectroscopy, physical chemistry of hydrogen and mercury vapour, the interaction of fast neutrons with atomic nuclei and the isomeric radioisotope of bromine.

After the War he became professor at Lodz Polytechnic (1945–47), returning to the University of Warsaw as professor of experimental physics (1947–48) and then as professor of atomic physics.

Dr. Soltan was a delegate at the Second International Conference on the Peaceful Uses of Atomic Energy at Geneva in 1958 and was a member of the State Council for the Peaceful Uses of Nuclear Energy.

NEWS and VIEWS

The Agricultural Research Council : Sir William K. Slater, K.B.E., F.R.S.

Sir William Slater will be retiring on June 30 from the secretaryship of the Agricultural Research Council, a post which he has filled with great distinction during the past eleven years. On graduating in chemistry at the University of Manchester, Sir William did research under Prof. Lapworth and for three years he was an assistant lecturer in the University. After two years in industry, he joined Prof. A. V. Hill at Manchester to assist in his work on muscle physiology. In 1923 he was awarded a Beit Memorial research fellowship and worked in the Department of Biochemistry of University College, London, under Sir Jack Drummond, where he developed a pioneer line of research on the biochemistry and physiology of insects, particularly anaerobiosis. In 1928 he went to Dartington Hall as scientific adviser to the trustees. Afterwards, he took charge of the agricultural and rural development on the estate and established a laboratory for applied research. In 1943, Sir William joined the staff of the Ministry of Agriculture, Fisheries and Food as secretary of the Agricultural Improvement Council for England and Wales, which had just been set up. He became responsible for the development of that Council's work, and had much to do with fostering the close relationship which exists between it and the Agricultural Research Council. Later, he became in addition senior advisory officer, responsible for setting up the specialist scientific section of the National Agricultural Advisory Service. This broad experience qualified Sir William well for the post of secretary of the Agricultural Research Council to which he was appointed in 1949. During his term of office he has brought to a successful conclusion a ten-year post-war programme for the development of agricultural research approved by the Cabinet in 1945. In 1947–48 the amount spent by the State on agricultural research was of the order of one million pounds ; to-day it amounts to about six million pounds. New institutes have been created and existing ones have been expanded. Links with universities have been strengthened by the establishment of a number of units in university departments. The Council has indeed been fortunate in having the services of Sir William Slater over this period, and his wise guidance and never-failing patience will be missed.

Prof. E. G. Cox, F.R.S.

Prof. Ernest Gordon Cox, who will be succeeding Sir William Slater as secretary of the Agricultural Research Council on July 1, 1960, comes originally from Somerset. He was born at Bath in 1906 and educated at the City of Bath Boys' School and the University of Bristol, from which he graduated with first-class honours in physics in 1927. From Bristol he went as research assistant to Sir William Bragg at the Royal Institution during 1927–29 and there began the series of researches which transformed his formal field of interest from physics to chemistry. During 1929–45 he held appointments in the Department of Chemistry of the University of Birmingham, and in 1945 was appointed professor of inorganic and physical chemistry at the University of Leeds ; his title was changed in 1953 to professor of inorganic and structural chemistry in a perhaps hopeless attempt to define more precisely the many-sided implications of his researches. He was one of the first to apply X-ray methods to solve problems in the stereochemistry of metal co-ordination compounds. But he has also made many contributions in organic chemistry, from his early work on α-chitosamine hydrochloride and hydrobromide, which established, by direct X-ray analysis, the existence and form of the pyranose ring in sugars, to his latest, very accurate, studies, with D. W. J. Cruikshank and others, of the electron distribution in benzene. In recent years his School of Chemistry has made Leeds an international centre to be visited by all interested in exact X-ray analysis. At the same time he has had much to do with problems of organization and administration, first in war research and later, in Leeds City and University ; for the last three years, as chairman of the X-ray Analysis Group, he has been concerned with the practical applications of X-ray methods in many different fields. His variety of experience and knowledge, his own personal interest in agricultural problems (he is a serious amateur botanist and ornithologist) and his generous spirit should be of the greatest value in guiding agricultural research.

Building Science at Sheffield : Prof. J. K. Page

Mr. J. K. Page, at present lecturer in environmental physics in the Department of Building Science of the University of Liverpool, has been appointed to the newly created chair of building science in the University of Sheffield. Mr. Page was educated at Haileybury College and the University of Cambridge, where he gained first-class honours in Part I of the Natural Sciences Tripos. After graduation, Mr. Page held a temporary appointment with the Council of Industrial Design, where he assisted with plans for the Festival of Britain. After teaching at Westminster School for two years he took an appointment as senior scientific officer in the Colonial Liaison Section at the Building Research Station. During this appointment Mr. Page travelled extensively in West Africa, America and the West Indies. After a period as chief research officer of the Nuffield Foundation Division of Architectural Studies, Mr. Page was appointed to his present post in the University of Liverpool in 1957.

Royal Astronomical Society Awards

Awards of medals have been made to the following by the President and Council of the Royal Astronomical Society : *Gold Medal* to Prof. V. A. Ambartsumian, president of the Academy of Sciences and director of the Byurakan Observatory, Armenia, for his numerous important contributions to both theoretical and observational astronomy ; *Eddington Medal* to Dr. R. d'Escourt Atkinson, deputy chief scientific officer at the Royal Greenwich Observatory, for his pioneering papers on atomic synthesis and stellar energy ; *Jackson Gwilt Medal and Gift* to Mr. F. Malne Bateson, of Cook Islands, and Mr. A. F. A. Lofley Jones, of Timaru, New Zealand, for their work on variable stars in the southern hemisphere.

British Association: Installation of President

THE installation on January 8 of Sir George Thomson as president of the British Association for 1960 (see *Nature*, **184**, 151; 1959) marked the close of a year of increasing activity and encouraging and significant developments. The lecture service, in its infancy in January 1959, is now well under way. Some 500 scientists have agreed to give lectures, and their audiences run into five figures—around 22,000 adults from trade unions, rotary clubs, women's institutes and chambers of commerce, and 32,000 children from all types of schools. The three pioneer area committees of 1958 have been joined by twelve more; the last of these, in York, is a direct follow-up of the recent annual meeting there. The decision to make a number of fundamental changes in the quarterly journal of the Association was also taken during the past year. From May 1960 *The Advancement of Science*, in a completely revised format, will be published six times a year, instead of four. Though still containing papers delivered at the annual meeting, the journal will include much new material, and will be of an essentially non-specialist character, aimed to appeal to scientists working in other fields, students and sixth forms in the schools, and laymen who are anxious to understand something of the significance of scientific research and development. The wide potential circulation of such a journal should make it a powerful weapon in the Association's campaign.

Nuclear Energy Conferences and Symposia in 1960

THE International Atomic Energy Agency, Vienna, has issued a provisional schedule of meetings to be organized in 1960. One large conference will deal with Radioisotopes in Physical Sciences and Industry and will probably be held at the end of August. The last major isotope conference, apart from the sessions devoted to this subject at the Geneva Atoms for Peace Conference in September 1958, was organized in Paris in September 1957 by Unesco, which will co-operate in the 1960 isotope conference. Another scientific conference will deal with problems of medium and small-size reactors and is planned for the beginning of September in Vienna with the expected participation of the United Nations. The tentative list of symposia to which invitations will be extended to all the Agency's Member States is as follows: (1) Fuel Element Fabrication with Special Emphasis on Cladding Materials (May 3–6, in Vienna); (2) Radiation Dosimetry in Physical Sciences (June 7–13, in Vienna); (3) Effects of Ionizing Radiation on Seeds and its Significance on Crop Improvement (probably in Karlsruhe, August 8–12, in co-operation with the Food and Agriculture Organization); (4) Inelastic Scattering of Neutrons in Solids and Liquids (October 11–14, in Vienna); (5) Neutron Pile Research (October 17–21, in Vienna); (6) Chemical Effects of Nuclear Transformation (October 24–27); (7) Nuclear Ship Propulsion with Special Reference to Safety (mid-November; the arrangements will be worked out in consultation with the International Maritime Consultative Organization); (8) Experimental and Test Reactors (November 28–December 2, in Vienna); (9) Pest Control by Radiation (probably in India in December, in co-operation with the Food and Agriculture Organization); (10) Use of Radioisotopes in the Study of Endemic and Tropical Diseases (in the Far East at the end of November or beginning of December, in co-operation with the World Health Organization); (11) Education and Nuclear Energy (probably in Argentina at the end of the year or beginning of 1961, in co-operation with Unesco).

Scientific Man-power in Britain

IN an article, "The Statistics of Scientific Man-power", in the January 7 issue of *Education*, Sir Solly Zuckerman points out that the figures since published by the Scientific Man-power Committee, which were not available to Prof. J. Jewkes when he presented his analysis of the marked shortage of scientists before Section *F* (Economics) of the British Association at York last September, indicate that the conclusion drawn by Prof. Jewkes is wide of the mark. Graduates in pure science from British universities during 1956–59 numbered only 15,500, and not 18,000 as he stated, and even taking into account about 800 scientists, not considered by Prof. Jewkes, who were admitted to membership of the Royal Institute of Chemistry or the Institute of Physics on the strength of qualifications other than a university degree, when allowance is made for those who returned to their homes overseas or took up employment abroad (about 500) and for the 5,000 needed to replace losses, we are left with about 10,800 to satisfy any net increase in demand expected between 1956 and 1959 (11,700 in the 1956 estimate).

The Scientific Man-power Committee's recent report also shows that there were still about 4,000 vacancies at the beginning of 1959. The new estimates made by employers themselves of the number of scientists they will require by 1962 suggest that the general market shortage of scientists is still likely to be about 2,000 in 1962. Commenting further on Prof. Jewkes's 'working rule' that employers know best about their future requirements, Prof. Zuckerman also points out that the demand between 1956 and 1959 rose much more sharply than employers anticipated, and that in practically no branch of industry did action follow intention. We have still far to go before we need be unduly concerned about the exhortatory character of Prof. Jewkes's concept of 'unmet need'; but the Scientific Man-power Committee would certainly agree wholeheartedly with him that the criteria now available for estimating how many scientists and technologists will be needed in the long-term are very crude and urgently need to be refined.

Computation and Automatic Control

AT the annual general meeting of the British Group for Computation and Automatic Control of the British Conference on Automation and Computation, held on December 17, 1959, the Executive Committee was reconstituted for 1960 as follows: *chairman*, Mr. J. F. Coales; *vice-chairmen*, Mr. R. Hindle and Mr. H. W. G. Gearing; *honorary treasurer*, Mr. S. M. Rix; *honorary secretary*, Mr. W. Bamford; *members*, Dr. A. D. Booth, Mr. N. Clarke, Mr. E. C. Clear Hill, Mr. W. C. F. Hessenberg, Mr. N. C. Pollock, and Prof. E. J. Richards. Groups *A* and *B* have undertaken the sponsoring of the British contribution to the First Congress of the International Federation of Automatic Control which is being held in Moscow during June 27–July 6. Further details can be obtained from the Honorary Secretary, Group *B*. Arrangements are being made also for a British Convention on this subject to be held in London in September, and further information can be obtained from the Honorary Secretary,

Group *A*. The address of both honorary secretaries is, the British Conference on Automation and Computation, c/o Institution of Electrical Engineering, Savoy Place, London, W.C.2.

Post Office Engineering-Apprenticeship Scheme

It was announced in Parliament by Miss Mervyn Pike, Assistant Postmaster General, that the Post Office is engaged in an expanding nation-wide programme of mechanization and automation. To enable it to carry this through, men will be required to train for key engineering-posts. A scheme is being launched under which university training or a diploma in technology course at a college of advanced technology will be guaranteed to about twenty students. During the student apprenticeship, including the time of attendance at university or college, salaries will be paid according to age, and varying from £270 per annum at seventeen to £430 at twenty-one and over. Student apprentices will be selected from young men of British nationality who are between the ages of seventeen and twenty on June 1 in the year when apprenticeship starts. The university or college studies will start in September/October of the year after apprenticeship begins. The student apprenticeship will last 4½ years, except in Scotland and Northern Ireland, where it will be slightly longer to allow for the duration of university courses. Further information can be obtained from the Engineering Chief (O.51), Post Office Engineering Department, 2–12 Gresham Street, London, E.C.2. Applications for 1960 must be submitted by February 26.

National Science Foundation : Science Faculty and Senior Postdoctoral Fellowship Awards for 1960

The National Science Foundation has awarded 285 Science Faculty Fellowships in the sciences for the fiscal year 1960. These fellowships are offered as a means of improving the teaching of science, mathematics and engineering in American colleges and universities, and their primary purpose is to provide an opportunity for college and university science teachers to improve their work as teachers. Candidates must have a first degree or its equivalent, demonstrated ability and special aptitude for science teaching and advanced training, and three years of full-time science teaching at the college-level. There were 762 applicants from all parts of the United States and its territories.

The Foundation has also announced the award of 75 Senior Postdoctoral Fellowships in the sciences for the fiscal year 1960, selected from 259 applicants. These Fellowships were awarded to scientists of demonstrated ability and special aptitude for productive scholarship in the sciences. Twenty-eight awards were made in the life sciences, and forty-seven in the physical sciences.

High Mach Number Air-breathing Machines

The Combustion and Propulsion Panel of the Advisory Group for Aeronautical Research and Development, North Atlantic Treaty Organization, will hold its fourth colloquium in Milan during April 4–9. The meeting will be devoted to discussions on "High Mach Number Air-breathing Engines". The opening session will consist of a round-table discussion on "The Future of Air-breathing Engines". Invited papers from Germany, Italy, the United Kingdom and the United States will deal with: (1) performance and applications, (2) hypersonic inlet studies, (3) supersonic combustion and detonation, (4) nozzle flow with chemical reactions, (5) rotating machinery, and (6) high-temperature material problems. Although the meeting is unclassified from a security point of view, attendance is limited to those who are authorized to attend by either the Group national delegates, or by members of the Combustion and Propulsion Panel.

Ford Foundation Grant to Cern

The European Organization for Nuclear Research has received from the Ford Foundation a grant amounting to five hundred thousand dollars to cover a period of four years. The facilities available at Cern now consist of two big particle accelerators, a 600 MeV. synchrocyclotron and a 28,000 MeV. proton synchrotron. The latter, the most powerful of its type in the world, has recently started operating with outstanding success. This grant follows a previous one of four hundred thousand dollars contributed by the Foundation in 1956 to cover similar expenses during four years.

Announcements

It is announced that Cornelius Lanczos, senior professor in the School of Theoretical Physics at Dublin Institute for Advanced Studies, has recently been awarded the Chauvenet Prize by the Mathematical Association of America for an article entitled "General Theory of Linear Differential Operators". This article is to form the last chapter of a book which is to be published by Van Nostrand, entitled "Linear Differential Operators", by Prof. Lanczos.

Mr. P. N. Harvey has been appointed farm director at Gleadthorpe Experimental Husbandry Farm, Notts., of the Ministry of Agriculture, Fisheries and Food, in succession to Mr. P. J. Jones, who has been transferred to Bridget's Experimental Husbandry Farm.

Prof. Michael Polanyi, emeritus professor of social studies in the University of Manchester, will give the 1960 Eddington Memorial Lecture in Cambridge at the Arts School Lecture Theatre, Bene't Street, on February 16, at 5 p.m. The title of his lecture is "Beyond Nihilism".

The McCrone Associates of the United States are sponsoring a National Microscopy Symposium which will be held at the Del Prado Hotel in Chicago during June 13–15. The Symposium will cover (1) Instrumentation, (2) Methods and Techniques, and (3) Applications. Further information can be obtained from Walter C. McCrone Associates, 501 East 32nd Street, Chicago 16, Illinois.

Nos. 7, 8 and 9 of the Bibliography of Scientific Publications of South and South-East Asia, for July, August and September 1959, respectively, compiled jointly by Unesco Science Co-operation Offices for South and South-East Asia and published by Insdoc, National Physical Laboratory, New Delhi, contain a further 1,660 classified entries, together with author index, and the August issue includes also the Index to Vol. 4 (1958).

Erratum. In the communication entitled "Isolation of 2-Aminoethane Phosphonic Acid from Rumen Protozoa", by M. Horiguchi and M. Kandatsu, in *Nature* of September 19, p. 901, Fig. 1 has unfortunately been inverted.

THE WILDFOWL TRUST

THE report* of the Wildfowl Trust for 1957–58 describes the administrative activities of the staff, the state of the accounts (satisfactory), the growth in membership (from 5,094 in 1957 to 5,320 in 1958), the work of scientific members of staff during the year, as well as investigations and observations of wildfowl by many well-known correspondents throughout the world. One of the latter is an interesting account by Eric Fabricius, of the University of Stockholm, of the mechanisms which make plumage waterproof. Fabricius found that ducklings of several species lost their waterproofing on a diet consisting of raw crushed fish, and regained it on a diet of insects; even in specimens that had lost their waterproofing there were indications that the oil gland was functioning.

The oil gland was removed from fourteen young tufted ducks just after hatching, while their down feathers were still enclosed in their isolating horny sheaths. After bursting of these sheaths and the full development of the down, the plumage was as repellent to water as that of the normal control birds, indicating that it is not the secretion of the oil gland which makes the plumage waterproof.

Operated and normal control birds alike lost their waterproofing when subjected to contact with smearing substances. In this condition the barbules of the down feathers were disarranged, sticking together in groups, between which were irregular spaces. The normal waterproofing was gradually restored if the birds were allowed to preen. Plumage remains water-repellent when birds feed on insects because insects are dry and clean. Plumage even remains water-repellent on a diet of fish, provided that the pieces of fish are only given to the birds while these are swimming in clean water. The preening consists of a number of stereotyped movements. The nibbling type of preening movements, in particular, aid in maintaining the barbules in a state which makes the plumage repellent to water. These findings confirm the view of Marsden that the large amount of finely distributed air among the ramifications of the feathers is the principal factor in the water-repellency of plumage.

* Tenth Annual Report of the Wildfowl Trust, 1957–1958. Edited by Peter Scott and Hugh Boyd. Pp. 184+32 plates. (Slimbridge, Glos.: The Wildfowl Trust, 1959.) 10s. net.

The causes of death of birds at Slimbridge during 1955–57 have been analysed by A. R. Jennings, of the University of Cambridge. Of the 680 birds examined, the largest number of deaths were due to the high incidence of acute pulmonary congestion and pneumonia among very young birds. Sudden lowering of the air temperature often associated with heavy rainfall brings about this pulmonary syndrome, which is often referred to as 'chilling'. This type of acute respiratory disease is a common cause of high mortality in free-living species, and the same conditions seem to apply even under conditions of semi-captivity. The changes at post-mortem are quite typical and comprise acute congestion and œdema of the lungs, an enlarged gall bladder full of viscous bile and usually an unabsorbed yolk sac. In all, 138 birds died from various infections. Aspergillosis was the most frequent with 117 cases, the predilection sites for the fungus being the lungs and air sacs. The lesions were usually typical fungal granulomata, but in some birds there was an acute diffuse pneumonia. Tuberculosis was the next most common disease, and eight cases were seen.

One of the main objectives of the research programme at Slimbridge has been to obtain a better understanding of the taxonomy of the Anatidæ. All the work has now been surveyed by Dr. G. U. T. Matthews, the assistant director of the Trust, who shows that a whole variety of techniques are open to the modern taxonomist. Many of these techniques are incapable of assessing relationships below the family level and are only of passing interest when concerned with relationships within a family. It is beginning to appear that the Anatidæ is an unusually homogeneous and closely knit family.

What makes the report outstanding, of course, is the revealed and hidden imprint of Peter Scott, the director. His sketches lose none of their attractiveness with the years; they may even excite more interest as they become more audacious. The many photographs are included only if they illustrate the work of the Trust and do so with adornment; not one of them is below standard. With a report of such high quality it is to be hoped that the activities of the Inland Revenue authorities may not, as is feared, lead to the production of future reports in an attenuated form.

DAIRY RESEARCH IN SCOTLAND

THE recent triennial report of the Hannah Dairy Research Institute* gives a clear picture of the expansion of the Institute's activities in the past three years. New laboratories have been built, and greatly increased facilities have been added to those formerly available for metabolic studies. The work reported continues to reflect the aims defined when the Institute was founded some thirty years ago.

Among several important additions to the Institute is a splendidly equipped climatic laboratory. This has been constructed, with capital provided by the Colonial Office, and is being used for studies on the effect of climate on the physiology of cattle. The laboratory includes a room in which a cow or steer can be maintained at any temperature from 0° to 60° C. within ± 0·1° C., and at a relative humidity within ± 1 per cent over a wide range. A second room houses four cattle with slightly less critical control of environment. Metabolism houses have also been constructed for sheep and cattle. These contain six new closed-circuit respiration chambers for sheep and one for cattle, and provision is also made for

* Hannah Dairy Research Institute. Report for the Three Years ended March 31, 1959. Pp. 52.

twenty-four sheep metabolism cages. With their unique equipment and instrumentation the climatic and metabolism buildings are a notable example of collaboration between biologists, physicists and engineers.

The Grass and Dairy Husbandry Department has continued its investigation of the intensive management of a largely grassland dairy farm. Purchases of fertilizer rather than concentrates continue to show a high return, and the Ayrshire herd averaged 800 gallons a year with one cow per 1·8 acre. The choice of forage species and strains, coupled with suitable management and fertilizer treatments, is being studied in extensive plot and feeding experiments. In the Biochemistry Department fundamental studies are in progress concerning the chemistry of milk protein and the vitamins and enzymes of milk. Attention has been given to factors influencing the important riboflavin and vitamin A components of milk, to the synthesis of lysine in the rumen and to the effect of thyrotropin and growth hormone on milk yield.

The construction of new equipment has not prevented the Nutrition Department from continuing its important studies on energy metabolism. These have shown that above the maintenance-level the utilization of mixtures of volatile fatty acids by sheep decreases with decreasing chain-length, being as low as 30 per cent with some mixtures rich in acetic acid. In ruminants almost all dietary carbohydrate is absorbed as these short-chain acids, and world-wide interest has been aroused by this first step in the reconciliation of classical energy studies with recent developments in ruminant nutrition.

Other work with sheep has been concerned with the effects of environmental temperature and clipped fleeces on energy metabolism, the influence of the physical state of roughages on their net energy and the role of vitamin E in muscular disease. Cow-feeding experiments suggested that the allowance of concentrates per gallon of milk should increase at higher yields.

The Physiology Department is concerned with the physiological responses of cattle to climatic stress. While aimed at the improvement of cattle productivity in hot countries, this work must also provide fundamental information of value in studies on housing in Britain. Two small chambers have been used for investigating the physiology of panting; the thermal stimulus to panting was found to be effective, in calves, only on the skin or upper respiratory tract. The ability to reduce tidal volume with increasing thermal stress appeared to be associated with high heat-tolerance as indicated by rectal temperature.

The validity of rectal as a measure of deep body temperature, the effects of heat stress on the blood and the role of the skin in loss of heat were also investigated. Important studies on the rate of acclimatization of calves to heat are now being extended to cows.

The Department of Veterinary Pathology studies both practical and applied aspects of mastitis. Histological examination of udders from infected cows, the incidence on farms of different types of mastitis and the relationship between the Whiteside test and cell counts have all been considered.

The Bacteriology Department has been largely concerned with the important *coli-aerogenes* bacteria of milk and with the application of the coliform test. Demand for sterilized milk in cities has prompted investigation of the spore-forming bacteria of such milk and of tests for effective 'sterilization'.

Many practical aspects are also dealt with by the Department of Technical Chemistry. Welcome collaborative studies are reported on the accuracy of various analytical methods for milk, and these have led to revision of the formula for the calculation of solids-not-fat content by means of the lactometer. Feeding trials indicated that with spring grazing the usual increase in milk protein is probably due more to an improved plane of nutrition than to an increase in any particular component of grass. Long-term studies have dealt with factors influencing the coagulation of casein, a problem in the manufacture of dairy products, and with the production, preservation and storage of dried milk. A new system was developed for the fractionation of the carbohydrates of roughages.

Readers of this report are provided with a ready reference to more detailed information by the liberal inclusion of relevant reprint numbers.

C. C. Balch

CORRELATION OF AUDIO-FREQUENCY ELECTROMAGNETIC RADIATION WITH AURORAL ZONE MICROPULSATIONS

By Dr. J. AARONS

Electronics Research Directorate, Air Force Cambridge Research Center

AND

G. GUSTAFSSON and A. EGELAND

Kiruna Geophysical Observatory

During three periods of approximately one month each, continuous recordings were taken of the electromagnetic energy in the spectrum region of 10 c.p.s. to 10 kc.p.s. The detection equipment was a 10-m. vertical antenna, a swept frequency battery-operated spectrum analyser and a recording milliammeter. The frequency analyser had two ranges; it first swept through its lower range of 10–1,000 c.p.s., with a 1 c.p.s. band-width. A second sweep was then initiated to go from 500 c.p.s. to 10 kc.p.s. with a 40-c.p.s. band-width. A total time for a complete recording of both of the sweeps was 32·8 min. The equipment was housed at an isolated area about 2 km. from the nearest 50 c.p.s. power line. An example of the registration of the higher-frequency range is given in Fig. 1.

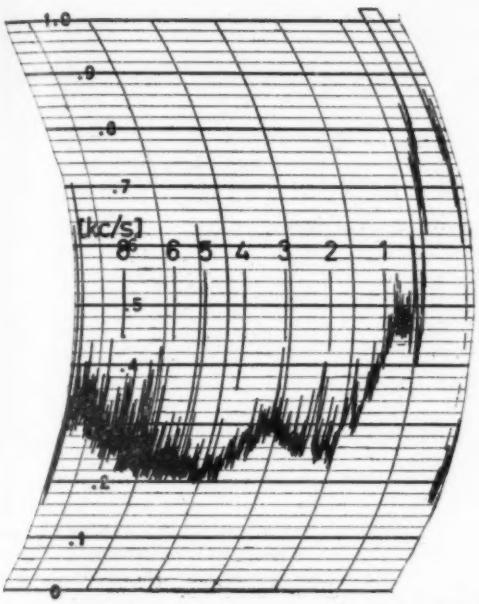

Fig. 1. Recording of the electromagnetic radiation of December 5, 1828 to 1839 M.E.T., 1958, in the frequency range 0·5–10 kc./s.

The aim of the programme carried out near the Kiruna Geophysical Observatory, Sweden (geographic co-ord., 67·8° N., 20·4° E.; geomagnetic co-ord., 65·3° N., 115·5° E.), was two-fold, namely: (1) to investigate changes in the background signal-level; (2) detect emission or absorption of energy at the gyro-frequency of solar terrestrial ions.

Several magnetically disturbed periods occurred during the measurement periods October 12–November 11, 1958, December 12, 1958–January 11, 1959, and April 15–May 14, 1959. On eleven occasions electromagnetic radiations associated with micropulsations of the Earth's magnetic field were detected. Two distinct frequency-bands were identified in this low-frequency emission. The lower-frequency emission, in the range 0·5–1·4 kc./s., has been mentioned by Egeland[1] as "noise" during aurora, while the higher-frequency emission, in the range 1·8–4·5 kc./s., was identified by Watts[2] as "hiss". For the lower-frequency emission very little results have hitherto been obtained.

Fig. 2 is a spectrum analysis of the signal received on December 5, 1958. For 13 hr. from 0701 to 2010 M.E.T. a distinct signal was seen in one or both of the two bands. Since the results are recorded in the form of a spectrogram, only the frequency variations are shown here. The cross at the centre of each sweep denotes the frequency of maximum intensity, while the length of line indicates the frequency-width of the emission above that of the background signals. For these sweeps the 'hiss' signals maxima varied from 2·3 to 3·4 kc./s. By contrast, the lower-frequency emissions do not vary more than + or − 150 c.p.s. around 750 c.p.s. The band-width of the lower-frequency emissions is less than that of the upper band, as can be seen on the spectrograms in Fig. 2. The error of reading and the band-width of the equipment lead to a value of + or − 100 c.p.s. for the accuracy of the measurements. Thus, while the lower-frequency band remained fixed in frequency the 'hiss' emissions varied. The amplitudes of each of the bands fluctuated from sweep to sweep during every disturbed period.

In all the eleven observed periods of emissions, micropulsations of the magnetic field were shown on the Kiruna magnetograms. The micropulsations seen in the auroral zone show periods of the order of 0·5–5 min. and maximum displacement of 50 γ. These do not occur during the largest deflexions of the magnetic storms. In four of the longer periods of radiation detected, the low-frequency emissions occurred between two phases of a magnetic storm when micropulsations were present.

The Kiruna magnetogram for December 5 is shown in Fig. 3. A tracing of the outlines of six consecutive recordings during the December period is shown in Fig. 4. The time of duration of each tracing is about 10 min. The low-frequency tracing is not included, and therefore there is a gap of 23 min. between the recordings. The dotted line is the no-signal receiver noise line. The curvature of the zero line is due to equipment parameters. The vertical amplitude scale is logarithmic. The signals are at best of extremely low amplitude, rarely exceeding 5 μV. from the antenna.

The 'hiss' signals received have been observed before, but at lower latitudes. Watts[2] observed 'hiss' for 12 hr. at a site near Boulder, Colorado. The duration of our longest period recorded is somewhat longer than this. The presence of so many examples of this low-frequency emission during the short period of recordings indicates its prevalence in the auroral zone during periods of magnetic activity of the micropulsation types.

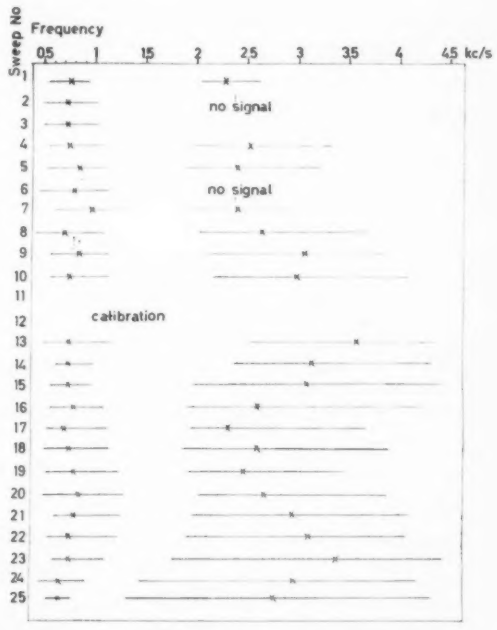

Fig. 2. A spectrogram of the signal received on December 5, 0701 to 2010 M.E.T., 1958. The length of line indicates the frequency-width. The cross on each line denotes the frequency of maximum intensity

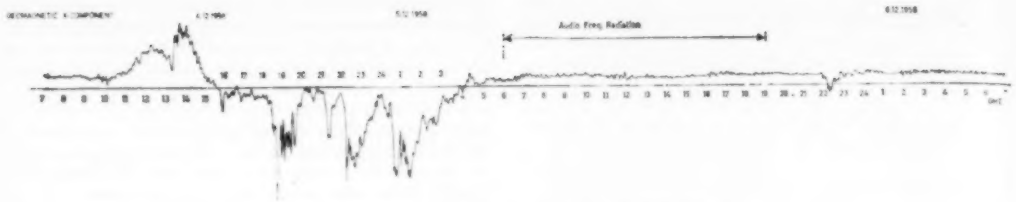

Fig. 3. The horizontal component of the geomagnetic field at Kiruna

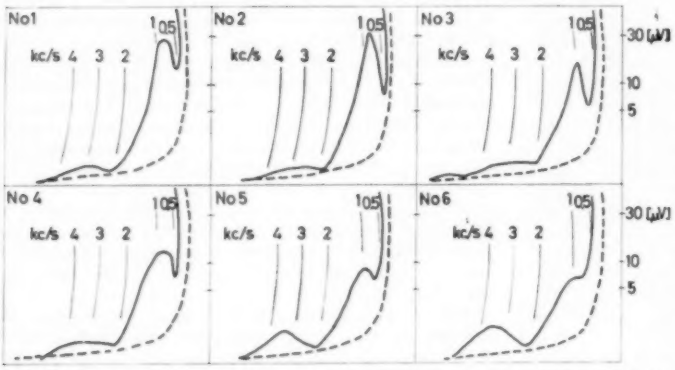

Fig. 4. Tracing of the outlines of six consecutive recordings of the December period 1958

One reason for being able to detect the signal is the low background-level of noise at the remote site and the low signal-level from atmospherics at Kiruna. Analysis of the three-months period (to be described in another publication) indicates that the normal diurnal variations of the atmospherics were not correlated with magnetic disturbances described in this article.

The lower-frequency emissions are of greater interest than the upper band principally because they lie for the most part near 750 c.p.s., the gyro-frequency of protons in the Kiruna area at an altitude of 100 km. Table 1 shows the gyro-frequency for protons in Kiruna as a function of altitude, calculated from the formula :

$$f_H = \frac{eH}{2\pi mc}$$

Fig. 2 demonstrates the stability of the lower-frequency emissions. However, on May 5, 1959, an emission band centred at 1 kc./s. was detected. In subsequent sweeps the single band began to split into two bands with the lower one slightly above 1 kc./s. and the upper band moving to 2 kc./s. Tracings of the signal are seen in Fig. 5. If the May 5 case is another example of 'hiss' developing into two bands it does not mitigate the case for proton radiation. However, it may well be that the lower-frequency band is a component of the 'hiss'. Only additional experimental evidence will determine this. The largest part of the data shows signals near 750 c.p.s. with relatively small changes in frequency.

The lower-frequency sweep has not as high a sensitivity as the higher one. The results from this channel indicate, however, that the 750 c.p.s. emission is relatively wide in spectrum. A sharply defined signal would have increased the level considerably in the lower-frequency sweep. It increased it only slightly.

The origin of low-frequency emissions has been discussed by various authors who have widely differing ideas. Ellis[3] states that Čerenkov radiation is the probable cause of 4–5 kc./s. auroral signals, Gallet[4] and Gallet and Helliwell[5] base their theory on an analogy to travelling-wave-tube amplifiers with modifications to suit the solar–terrestrial parameters and Aarons[6] compares the gas discharge mechanism with the aurora. Discussions of the possibility of detecting the protons and the other ions by the emissions is also discussed by Aarons.

Table 1

Altitude	Kiruna
0 km.	794 c./s.
60 km.	771 c./s.
100 km.	755 c./s.
200 km.	718 c./s.

The evidence in this article is rather clear. There are excellent correlations between the auroral zone micropulsations and the radiation in the audio-frequency band. Two distinct frequency bands have appeared on 12 per cent of the days for which results were obtained. This is a lower limit since on several days when the sensitivity was low, emissions were not detected. The upper-frequency band is 'hiss'. It varies in frequency throughout the recording period.

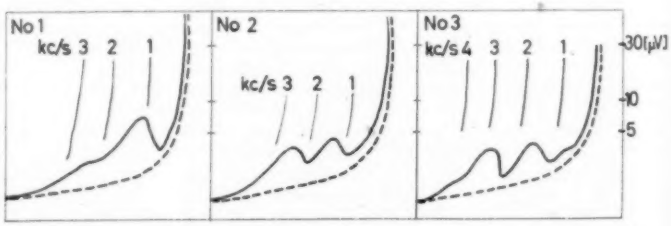

Fig. 5. Tracing of the outlines of three consecutive recordings of the May period 1959

The lower band at 750 c.p.s. for almost all the cases is steadier in frequency. Its spectral location indicates that it may well be radiation from protons, but its occasional change in frequency may make it another component of the 'hiss' signals.

We are indebted to Dr. B. Hultqvist, director of the Kiruna Geophysical Observatory, for his interest and advice.

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CELL-GROWTH OF PLANT ROOTS, INORGANIC SALTS AND NATIVE SERUM PROTEINS

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THIS article deals with the influence of increasing concentrations of some inorganic salts on the phytohormonal activity of native human serum compared with the influence of indolyl-3-acetic acid, which is considered the most important phytohormonally active substance in serum[1].

The phytohormonal activity of human serum, that is, its inhibitory effect on the growth of *Lupinus albus* roots, is influenced by the salt concentration in the nutrient solution with which the serum is diluted. Thus, the inhibitory effect of serum when diluted with distilled water is far less than when the serum is diluted with a plant-physiologically well-balanced triple-salt solution consisting of calcium nitrate 2·5 m*M*, magnesium sulphate 7·5 m*M*, and potassium dihydrogen phosphate 9·0 m*M* (after Shive[2])[3]. The increased effect of serum in a solution of salts, as the result of single-salt experiments, is interpreted as a phytohormone-separating effect of, especially, the calcium ions on the serum proteins[4].

The phytohormonal effect of serum is diminished, however, when the concentration of inorganic salts in a serum–salt solution is increased further, as will be accounted for below.

Fig. 1 shows the results of four, mutually independent, experiments (I, II, III and IV) with the growth of *Lupinus albus* roots in nutrient solutions, partly with and partly without admixture of native serum at the concentrations 3, 2, 2 and 1 per cent (v/v). The nutrient solutions used with or without the addition of serum comprise distilled water, Shive's solution, and solutions of the individual salts contained in Shive's solution. 1/1 × Shive thus denotes the salt solution specified above. 2/1, 3/1 and 5/1 × Shive denote salt solutions in which all the salt components are present at concentrations 2, 3 and 5 times that of 1/1 × Shive's solution. The upper curves of Fig. 1 show the growth in solutions without serum; the lower curves show the growth in solutions with serum.

The osmotic pressure in Shive's solution is about 1·3 atmospheres. Consequently, 5 × Shive has an osmotic pressure of about 6·5 atmospheres, corresponding to the osmotic pressure of undiluted human serum. It is a familiar fact that the osmotic pressure of serum proteins is extremely low: about 0·033 atmospheres.

In Fig. 1 the salt concentrations are shown on the axis of abscissæ, while the axis of ordinates shows the elongation of the roots in mm. per 24 hr. at 15° C. (the Macht–Livingston technique[5]). The salt concentration 0 in experiments I and II indicates that in these experiments distilled water has been used,

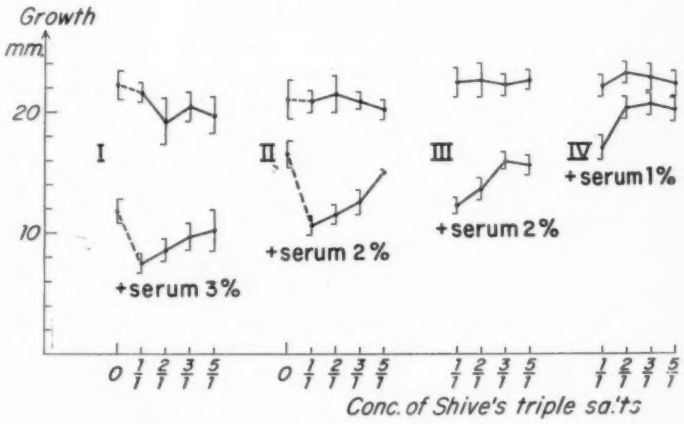

Fig. 1. Experiments I, II, III and IV. The phytohormonal effect, that is, the root-growth-inhibitory effect, of native, human serum at concentrations of 3, 2, 2 and 1 per cent (lower curves). For comparison, the upper curves show growth in the same solutions without serum, that is, in distilled water (experiments I and II only), in Shive's triple-salt solution, and in solutions with all the Shive salts multiplied by 2, 3 or 5 (experiments I to IV). Axis of ordinates: growth of roots in mm. in 24 hr. at 15° C.

partly with and partly without admixture of serum as nutrient solution.

The upper curves of Fig. 1 present the elongation in the above-named salt solutions and in distilled water without serum, respectively. It will be seen that in all solutions with serum the growth is unaffected irrespective of whether the salt concentration is one or five times the concentration in Shive's solution. In 24-hr. experiments the elongation in distilled water (the first two values on the upper curves for experiments I and II) is 0–10 per cent greater than in Shive's solutions[5,6].

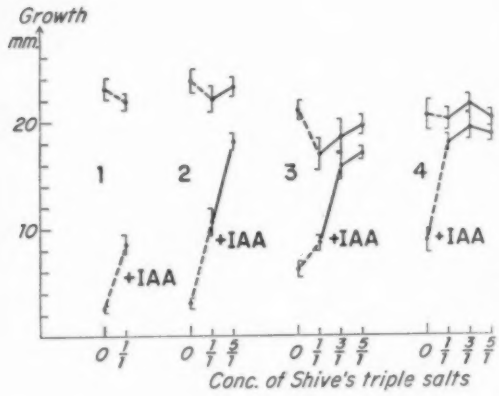

Fig. 2. Experiments 1, 2, 3 and 4. The phytohormonal effect, that is, the root-growth inhibitory effect, of indolyl-3-acetic acid (IAA) 10^{-7} *M* (lower curves). Upper curves show comparative growth in the same solutions without indole-3-acetic acid, that is, in distilled water, in Shive's triple-salt solution, and in triple-salt solutions with all the Shive salts at concentrations multiplied by 3 or 5. The concentration in Shive's solution alone is denoted by 1/1. Axis of ordinates: growth of roots in mm. in 24 hr. at 15° C.

From the first two values on the lower curves, representing the results of experiments I and II, it will be seen that in the solutions containing serum diluted with distilled water the elongation is greater than in the serum solutions diluted with Shive's salts. The growth depression brought about by the admixture of Shive's salt to the serum solution is accounted for in detail in a previous paper[4], and as mentioned, it is due to a specific serum-protein/inorganic-salt interaction.

In the solutions containing serum diluted with the various Shive salts, represented by the four lower curves in Fig. 1, which give the results of experiments I–IV, it will be seen that the elongation increases with a salt concentration increasing beyond the concentration in 1/1 × Shive. In all the experiments the elongation in the serum solutions containing Shive salts is thus lowest when the solution is diluted with 1/1 × Shive.

How, then, is this reduced phytohormonal activity of serum with increasing inorganic ion concentration to be interpreted?

It is impossible to give an exhaustive explanation of this phenomenon; but a partial explanation may be found by comparing the effect of increasing salt concentrations on a pure, unbound phytohormone in a corresponding experimental series and the effect just named of the same salts on the protein-bound phytohormone in human serum.

Fig. 2 serves this purpose. It shows the results of four experiments (1, 2, 3 and 4), in which indolyl-3-acetic acid is dissolved, partly in distilled water, partly in Shive's solution, and partly in solutions containing increasing concentrations of the individual Shive salts, as described previously for Fig. 1. From the upper curves for the four experiments in Fig. 2 it will be seen that, as was the case in Fig. 1, the elongation in the salt concentrations without indolyl-3-acetic acid is, in all essentials, constant with increasing salt concentration (experiment 3 of Fig. 2, however, shows a slight increase with increasing salt concentration), while, as previously, the elongation in distilled water with indolyl-3-acetic acid is greater than, or of the same order as, the elongation in the salt solutions.

From all the lower curves in Fig. 2 it will be seen that the root-growth inhibition, the phytohormonal activity, of the unbound indolyl-3-acetic acid in an aqueous solution at the concentration 10^{-7} *M* is greatly pronounced but decreases rapidly when the indolyl-3-acetic acid solution contains a certain amount of inorganic salts (with calcium ions). This effect is not known to have been described before for the root-growth experiments, and it is preponderantly due to the presence of calcium ions in the solutions[4]. For *Avena* coleoptiles, Thimann and Schneider[7] have described a similar calcium–auxin antagonism in single-salt experiments with calcium chloride. The increased auxin-induced elongation of coleoptiles was thus inhibited by the addition of calcium at concentrations above 0·001 *M*. Through experiments, likewise with *Avena*, Cooil and Bonner[8] demonstrated that this calcium effect is associated with a tightening effect upon the cell wall and is apparently brought about through interference with the normal processes involving auxin.

Like coleoptile cells, also plant root cells become apparently more resistant to action of externally applied phytohormones with increasing calcium concentrations in the nutrient solution. The effect of increasing concentrations of the Shive salts beyond the concentration in 1/1 × Shive in the serum dilutions, illustrated in Fig. 1, may therefore be interpreted as being due to such an increased resistance of the roots.

Fig. 1 thus shows two salt or calcium effects, namely: (1) a primarily acting salt or calcium effect, acting on the serum proteins[3] and becoming apparent by a comparison of the phytohormonal effect (root-growth inhibition) of serum diluted with distilled water and the phytohormonal effect of serum diluted with 1/1 × Shive's triple-salt solution; (2) a secondarily acting salt or calcium effect, acting on the plant root cells and becoming apparent by an additional supply of Shive salts or calcium ions to the serum solution beyond the concentration in 1/1 × Shive.

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MICROBIAL OXIDATION OF GLYCOLLATE VIA A DICARBOXYLIC ACID CYCLE

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ALTHOUGH the tricarboxylic acid cycle[1] has been established as the major route for the oxidation of acetate, and of compounds catabolized to acetate, in micro-organisms as well as in other biological materials (for review, see Krebs[2,3], Elsden[4], Ajl[5] and Kornberg[6]), little is known of the pathways of oxidation of C_2-compounds more highly oxidized than acetate (such as glycollate, glyoxylate or glycine). Studies with mammals[7], with mammalian tissue preparations[8–10] and with a strain of *Pseudomonas*[11,12] have led to the suggestion that glycollate or glycine is oxidized via glyoxylate and formate (1):

$$\begin{matrix}CH_2.OH.COOH \\ \text{or} \\ CH_2NH_2.COOH\end{matrix} \xrightarrow{+O_2} CO_2 + HCOOH \xrightarrow{+\frac{1}{2}O_2} CO_2 + H_2O \qquad (1)$$

Evidence for the occurrence of this pathway in *Pseudomonas* rests largely on the demonstration that formate, which is rapidly oxidized, accumulates[11,12] when cells oxidizing glycine or glyoxylate are poisoned with hypophosphite[13].

However, the oxidation of glycollate and of glyoxylate by washed suspensions or cell-free extracts of *Pseudomonas aeruginosa*[14], of *Pseudomonas ovalis* Chester[15], or of *Escherichia coli* (strain *w*) proceeds at identical rates and to identical extents in the presence or absence of hypophosphite, although formate oxidation is completely abolished by this inhibitor. This indicates that a sequence of reactions (1) involving free formate is probably not of major importance in glycollate oxidation in these micro-organisms.

The observed effects on glycollate metabolism of other metabolic poisons, particularly of fluoroacetate[16,17], suggest that the metabolism of glycollate (and of glyoxylate derived therefrom) is linked to that of intermediates of the tricarboxylic acid cycle. One such link has been demonstrated[18]: extracts of glycollate-grown *Pseudomonas ovalis* Chester can catalyse the formation of intermediates of the tricarboxylic acid cycle from glycollate or glyoxylate. Acetyl coenzyme *A* can be formed by a sequence of reactions the overall effect of which is:

$$2 \text{ glyoxylate} + \text{coenzyme } A \rightarrow \text{acetyl coenzyme } A + 2CO_2 + H_2O \qquad (2)$$

and can react with glyoxylate to form malate (3), catalysed by malate synthetase[19]:

$$\text{glyoxylate} + \text{acetyl coenzyme } A + H_2O \rightarrow \text{malate} + \text{coenzyme } A \qquad (3)$$

Malate can be oxidized to oxaloacetate[20], which can condense[21] with acetyl coenzyme *A* formed in reaction (2) to yield citrate. Since citrate can be metabolized further through the tricarboxylic acid cycle, this sequence of reactions might serve not only to provide carbon skeletons for the biosynthesis of cell constituents when micro-organisms grow on C_2-compounds more highly oxidized than acetate[18,22,23], but might also serve as part of the terminal pathway of oxidation of such C_2-compounds.

This point was tested with a mutant of *Escherichia coli*[24] devoid of the citrate-forming condensing enzyme[21] and hence unable to utilize acetate and unable to form citrate and glutamate. The results obtained show that although intermediates of the tricarboxylic acid cycle are required for growth, this cycle is not an obligatory pathway for the oxidation of glycollate. Glycollate oxidation can be effected

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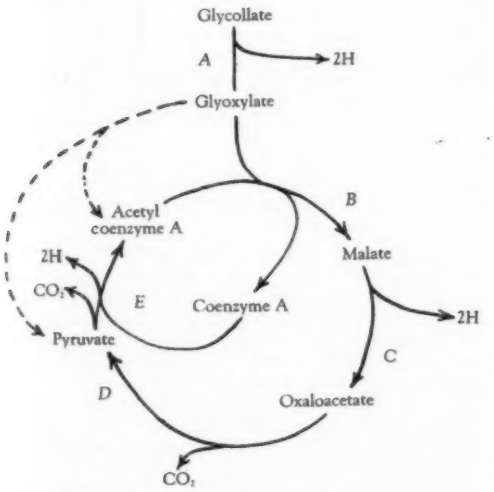

Fig. 1. Postulated dicarboxylic acid cycle for the oxidation of glycollate and glyoxylate. (The sequence of reactions is catalysed by *A*: glycollic oxidase (ref. 18); *B*: malate synthetase (ref. 19); *C*: malic dehydrogenase (ref. 20); *D*: oxaloacetic carboxylase (ref. 25) and *E*: the pyruvate oxidase system (ref. 26). Each turn of the cycle results in the oxidation of one molecule of glyoxylate: $CHO.COOH + O_2 \rightarrow 2CO_2 + H_2O$. The dotted line represents the pathways from glyoxylate to cycle intermediates, first shown by studies with *Ps. ovalis* Chester (ref. 18), which also operate in the strain of *E. coli* used in the present work, and which are a means of providing for the net formation of precursors of cell constituents during growth)

via a dicarboxylic acid cycle (Fig. 1), which obviates the necessity for reduction of glyoxylate to the oxidation level of acetate prior to its catabolism. It brings about the oxidation of glyoxylate (derived from glycollate[18], Fig. 1*A*) by condensation with acetyl coenzyme *A* to form malate[19] (reaction 3; Fig. 1*B*); oxidation of malate to oxaloacetate[20] (Fig. 1*C*); formation of carbon dioxide and pyruvate from oxaloacetate, possibly with intermediary formation of phospho-pyruvate[25] (Fig. 1*D*) and regeneration of the acetyl coenzyme *A* acceptor molecule from pyruvate by oxidative decarboxylation with the pyruvic oxidase system[26] (Fig. 1*E*). A major difference in principle between oxidations effected by the tricarboxylic acid cycle and those effected by the postulated dicarboxylic acid cycle is that, while in each turn of the former, one molecule of acetyl coenzyme *A* is completely oxidized with the obligatory participation of a keto-acid (which is regenerated), in each turn of the latter, one molecule of keto-acid is completely oxidized with obligatory participation of acetyl coenzyme *A* (which is regenerated).

The evidence for the cycle was obtained from studies on the growth of the *E. coli* mutant *M* 22–64[24], on oxidations effected by washed suspensions of the organism, and on enzymic reactions catalysed by extracts obtained after disintegration of the organism in a magnetostrictor oscillator. The main findings are as follows:

(1) The mutant grew readily on a medium containing glycollate (50 m*M*) plus glutamate (2 m*M*) as carbon sources, but did not grow on glycollate alone, or on acetate either in the presence or absence of glutamate. Growth on glycollate plus glutamate was stimulated both in rate and extent by the addition of acetate (2 m*M*). These results confirm[24] that glutamate, which is required for growth, cannot be synthesized by the mutant deficient in the ability to form citrate, and they support the view that the tricarboxylic acid cycle operates primarily to supply cell constituents under these conditions. Stimulation of growth by acetate indicated a point of entry into metabolic pathways of this compound other than via the condensing enzyme[21].

(2) Suspensions of washed cells oxidized glycollate plus acetate (Q_{O_2} = 158 μl. of oxygen absorbed/hr./mgm. dry wt.) more rapidly than glycollate alone (Q_{O_2} = 109), although they did not oxidize acetate alone. The addition of acetate, pyruvate or malate to cells oxidizing glycollate increased the quantities of oxygen absorbed from 45–59 to 83–93 per cent of that required for complete oxidation of glycollate. Pyruvate or malate were oxidized incompletely and the quantities of oxygen absorbed were consistent with their oxidation only to the level of acetate. The oxidation of glycollate was virtually complete in the presence of 2:4-dinitrophenol (1·3 m*M*).

(3) Washed suspensions of the mutant incorporated isotope from added [1-^{14}C] or [2-^{14}C]glycollate initially into malate, and thereafter into aspartate, alanine, phosphorylated compounds and sugars. No radioactivity was detected in glutamate, citrate or other intermediates of the tricarboxylic acid cycle.

(4) The addition of [2-^{14}C]acetate to cells oxidizing unlabelled glycollate resulted in the rapid appearance of the isotope in oxaloacetate and pyruvate. Similar results were obtained with [2-^{14}C]glycollate; labelled oxaloacetate and pyruvate accumulated in the presence of arsenite (10 m*M*).

(5) Washed suspensions of the mutant, shaken in air at 30°, did not evolve labelled carbon dioxide from [1-^{14}C] or [2-^{14}C]acetate alone, but did so rapidly if unlabelled glycollate or glyoxylate was added to the system. Malate or pyruvate was ineffective in promoting this oxidation of acetate.

(6) The rates and extent of evolution of labelled carbon dioxide from malate, labelled with carbon-14 in the carboxyl groups[27], were stimulated by the presence of glycollate or glyoxylate. This stimulation was abolished by arsenite (10 m*M*). Labelled oxaloacetate and pyruvate were formed in these reactions. Pyruvate or acetate was ineffective in stimulating release of labelled carbon dioxide.

(7) Cell-free extracts of the mutant readily catalysed the formation of malate from acetyl coenzyme *A* and glyoxylate, the activity of the malate synthetase[19] being exceptionally high and sufficient to effect the formation of 20 μmoles of malate/hr./mgm. of soluble protein under the conditions of assay[28]. Acetyl coenzyme *A* was not split by the extracts when oxaloacetate was added, confirming[24] the absence of the citrate-forming condensing enzyme.

(8) The activity of malic dehydrogenase[20] in such extracts was sufficient to catalyse the oxidation of 70 μmoles of reduced diphosphopyridine nucleotide/hr./mgm. of soluble protein when incubated with this coenzyme and oxaloacetate.

(9) The cell-free extracts catalysed the anaerobic decarboxylation of oxaloacetate, the initial rate of release of carbon dioxide (7·8 μmoles/hr./mgm. of soluble protein) being doubled by the presence of adenosine triphosphate[25].

(10) Although the activity of reduced diphosphopyridine nucleotide oxidases was too high to permit a quantitative assay to be made of the pyruvate oxidase system[26], the cell-free extracts formed at least 9·9 μmoles of reduced diphosphopyridine nucleotide/hr./mgm. of soluble protein when incubated with pyruvate, glutathione, magnesium chloride, thiamin pyrophosphate and coenzyme *A*, which showed that acetyl coenzyme *A* could be readily formed from pyruvate by the extract. The identity of the formed acyl thiolester with acetyl coenzyme *A* was established by chromatography of the acyl hydroxamates[29].

The enzymic rates described under (7)–(10) above are more than sufficient to account for the observed rates of glycollate oxidation (3–4 μmoles/hr./mgm. dry wt.). Coupled with the findings on the growth of the mutant (1) and the oxidation of unlabelled and isotopically labelled substrates (2)–(6), these results support the assumption that the sequence of reactions outlined in Fig. 1 occurs in the mutant of *Escherichia coli* studied, and that this oxidative dicarboxylic acid cycle represents a quantitatively important route for the microbial oxidation of carbon compounds at the oxidation-levels of glycollate or glyoxylate.

Full experimental details will be published elsewhere.

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FUNCTIONAL PITUITARY TRANSPLANTS IN RATS

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IN a paper published a number of years ago[1] one of us presented evidence demonstrating that anterior pituitary transplants from infantile rats grafted into the anterior eye chamber of hypophysectomized adult males will often restore the functional activity of the testes. It is true that in these early experiments no histological study of the sella was undertaken, although only those animals from which the pituitary gland was removed as a whole, practically uninjured, were considered for further study.

More recently, fresh work by Courrier[2] and by Fry and Long[3] has been published indicating that the effect of the functional activity of pituitary grafts, not only on the testes but also on other end-organs, may be far greater than is attributed to them at the present time. Owing to the multiple dependence of the pituitary gland upon the hypothalamus, and to the role, recently revealed, that the former takes in the functional activity of the latter, the prevailing opinion to-day is that the anterior pituitary by itself is capable of very little of the functional activity by which this organ is characterized in its normal position (see refs. 4 and 5). Prompted by the work of Courrier and of Fry and Long, we have repeated our work on 38 adult male rats with strict observance of all control experiments including a large number of hypophysectomized controls, histological study of the sella, mating of the animals under experiment with normal females and in four cases enucleation of the eyes bearing the grafts (see Table 1). We have also enucleated the eyes of two normal rats weighing 100 gm., but with no effect upon the testicular tissue of the same. To the above rats another 12 animals should be added which received grafts from rats 18–22 days old and which are still alive (see Table 2).

Four to nine whole anterior pituitaries from males 13–22 days of age were grafted at different time-intervals in the anterior eye chamber and, as the necessity arose, under the kidney capsule. If the host animal responded to the first batch of grafts (usually three or four whole anterior pituitaries) no further grafts were given to it. Out of 38 grafted rats, 13 or 34 per cent either had their retracted testes returned into the scrotal sac or when freshly hypophysectomized animals were grafted, incipient involution was arrested, thereafter followed by a restoration that was close to normal.

Some of the definitive results so far obtained have been presented in Table 1. In Table 2 are collected the results for the animals that received grafts from rats 18–22 days old and are still alive. To the grafted rats that sired a normal female should be added two more animals which were killed, but belonged to a still different batch of rats. This raises the number of rats siring normal females to 11. As a matter of fact it seldom happens that, once the process of restoration of the testes becomes definitely stabilized, a grafted male fails to sire a female.

For the benefit of those who wish to go deeper into the problem of the functional activity of the pituitary grafts we should recommend the observance of the following points.

(1) *Mass of the grafted tissue.* When three whole anterior pituitaries of infantile rats are grafted into hypophysectomized hosts, the testes of the recipient animal may or may not respond to the grafted pituitary tissue. Some hosts failing to respond to the first batch of grafts will do so if a second batch is given to them. Some of the hosts that failed to react to a second batch may react if a third batch is grafted under the kidney capsule. These observations have led us to believe that there may be a threshold sensitivity on the part of the testicular tissue to the hormones manufactured by the grafted pituitary tissue. A certain percentage of the grafted rat

Table 1. Hypophysectomized Grafted Rats with Enucleated Eyes

No. of batch	No. of animals	Body-weight (gm.)			Weight of testes (gm.)		Result of mating to normal females	Size of litter	Remarks
		At hypophysectomy	Before removal of grafts	When killed	Left testis before removal of grafts	Right testis after removal of grafts			
V–58/59	1	110	180	150	0·800	0·158	+	6	—
VII–58	1	97	185	143	1·074	0·543	Not mated	—	Animal had to be killed because of illness
IX–58/59	1	100	203	140	1·212	0·155	+	10	—
IX–58/59	1	97	170	141	0·954	0·182	+	9	—

Table 2. Hypophysectomized Grafted Rats still under Experiment with Grafts obtained from Males 18–22 Days Old

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				Good	Can be palpated	Retracted	Mated	Sired normal females	
X–58/59	5	97	234	4	—	1	4	4	—
XI–59	2	102	242	2	—	—	1	1	—
XII–59	3	99	173	1	2 recovering		—	—	These animals belong to a batch recently subjected to experiment
XIII–58/59	2	102	235	1	—	1	1	1	First grafted two months following hypophysectomy

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LETTERS TO THE EDITORS

PHYSICS

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In connexion with parallel electron microscope work being done in this laboratory[2], we have recently performed experiments in which various keratin fibres (Lincoln wool, mohair, human hair and pig bristle) have been impregnated with mercury acetate or silver nitrate after they had been treated with thioglycollic acid to reduce the disulphide groups. With the mercury, we find considerable differences between the diffraction effects produced in the different fibres, and would add the third, fourth and sixth orders to the list of those which can sometimes be enhanced.

Our most significant observations, however, have been made on the Lincoln wool fibres containing silver, for in these we find a quite exceptional enhancement of all the orders from the third to the tenth. Assessment of the absolute intensity of the scattering is made difficult by the high absorption of the metal, which makes a considerable reduction in specimen size necessary if X-rays are to be transmitted with reasonable intensity. However, if the specimen size and exposure are adjusted so that the conventional large-angle photograph can be discerned, the intensity of the scattering at low angles (corresponding to Bragg spacings greater than 20 A.) is so great that details of the discrete reflexions tend to be obscured; when the exposure is adjusted to allow these details to be observed, the photographic film is completely blank at higher angles of scattering.

To a first approximation, therefore, we can say that the effect of the silver is to enhance the meridional reflexions for orders up to the tenth at the expense of the higher orders. This can only mean that the silver is spread out at fairly wide intervals along the fibre axis. When the polarization and Lorentz factors are taken into account, it turns out that the eighth-order reflexion is the strongest by a factor of 2·5 at least; the tenth, sixth and third orders are also strong, and the fourth, fifth, seventh and ninth are present but relatively weak. The absence of the higher orders and the very great intensity of the eighth can be interpreted to mean that the silver atoms are distributed with some regularity at intervals of approximately·25 A. along the α-helix.

Whether the silver is taken up within or on the surface of the microfibril is at present being investigated with the electron microscope, and preliminary results (Sikorski, J., unpublished work) suggest that, as occurs with osmium staining, the metal is for the most part located on and between the microfibrils rather than in them. Since the diffraction effect described is conditional upon the reduction of the disulphide groups, it is tempting to suppose that in some parts of the fibre, at least, the cystine residues occur at intervals of 25 A. along the axis; it may be relevant that a relatively strong eighth-order reflexion occurs in untreated human hair[3] and Lincoln wool[4], but only a weak one in porcupine quill[3], which has a much lower sulphur content.

We wish to acknowledge the financial support of the Wool Textile Research Council, which made this work possible.

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X-RAY diffraction photographs of various keratins taken with fine-slit collimators of high resolution display a rich series of meridional reflexions[1-3]. Their spacings may conveniently be regarded as orders of a macro-period of 198 A. with very few exceptions. Astbury[4] has used the spacings and intensities of reflexions reported by Bear and others, from porcupine quill tip, to derive a one-dimensional Patterson diagram, and to explore the possibility of finding the sequence of amino-acid residues in the protein chains of the crystalline portion of the keratin. It might well be that the differences among keratins could provide useful clues in this quest, and the present intention is to direct attention to them.

Photographs were taken using copper $K\alpha$ radiation in an evacuated camera at specimen-film distances of

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Order of 198 A.	Bear, Astbury	Group *A*	Group *B*	Group *C*
3	6	6	6	6
4	1	2	1	2
5	2	1 (38 A.)	2 (38)	0
6	0	0	2	0
7	4	5 (27·0)	5	3
8	2	3	5	6
9	2	1	1	1
10	4	5	5	4
11	3	5	0 or 1	0 or 1
12	0	1	1	0 or 1
13	1	2	2	2
14	0	1 (13·9)	0	0
15	1	3	1	1
16	4	5	3	3*
17	0	0	0	0
18	0	2	0	1
19	3	2	2	2

* Pig bristle 4, human hair 2, horse hair 3.

Table 1. Hypophysectomized Grafted Rats with Enucleated Eyes

No. of batch	No. of animals	Body-weight (gm.)			Weight of testes (gm.)		Result of mating to normal females	Size of litter	Remarks
		At hypophysectomy	Before removal of grafts	When killed	Left testis before removal of grafts	Right testis after removal of grafts			
V–58/59	1	110	180	150	0·800	0·158	+	6	—
VII–58	1	97	185	143	1·074	0·543	Not mated	—	Animal had to be killed because of illness
IX–58/59	1	100	203	140	1·212	0·155	+	10	—
IX–58/59	1	97	170	141	0·954	0·182	+	9	—

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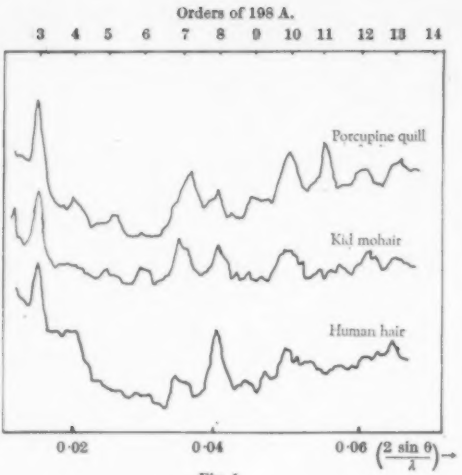

Fig. 1

24 and 20 cm. The six forms of α-keratin studied gave photographs that fall into three groups: group *A*, porcupine quill tip; group *B*, Lincoln wool and kid mohair; group *C*, human hair, horse hair and pig bristle. The intensities of the reflexions observed are reported in Table 1, using Bear's scale of intensities. In exceptional cases the spacing is given in brackets where the reflexion is displaced from the position appropriate to an order of 198 A.

It will be noted that the intensities for group *A* (porcupine quill tip) do not differ greatly from those reported by Bear and used by Astbury; the intensities for group *C* show differences in detail from those of Bear and Rugo, although the general trend is similar. The chief differences among the present groups are in orders 5, 6, 7, 8, 10, 11, 15 and 16. The intensities, similarities and differences observed by eye are confirmed by photometer traces, of which Fig. 1 shows typical examples. Further work is proceeding to investigate the possibility of extending the tentative classification used here to other varieties of keratin fibres.

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Thermo-magnetic Effects in Liquids

Recently, several authors[1–7] have investigated the effect of a magnetic field on thermal convection in electrically conducting liquids. In this communication we present the results of a simple experiment on the effect of the magnetic field on the rate of cooling of two liquids, namely, mercury and water. A glass tube (length 20 cm. and diameter 4·2 cm.) was placed on a frame suspended between the pole-pieces of a large electromagnet (type *ADL* Cambridge, Mass, U.S.A.). The frame was made of wood and brass rods, and was completely isolated (thermally) from the pole pieces of the magnet. The glass tube, filled with mercury up to a height of 15·7 cm., was heated and then allowed to cool in a magnetic field of about 23,000 œrsteds and without the magnetic field. The cooling curves for both cases are shown in Fig. 1. These curves clearly show that the rate of cooling for mercury is faster in the magnetic field. The experiment was later repeated also with water, and a similar effect on the rate of cooling was observed (Fig. 1).

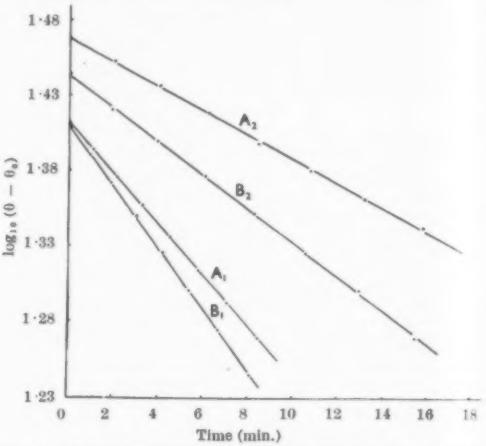

Fig. 1. Cooling curves for mercury and water with and without the magnetic field. Curves A_1 and B_1 refer to mercury, while A_2 and B_2 are for water. Curve *B* corresponds to the cooling in a magnetic field of ~ 23,000 œrsteds and *A* refers to field free cooling. Abscissa denotes time in minutes and ordinate $\log_{10}(\theta - \theta_s)$, where θ is the instantaneous temperature of the liquid and θ_s is that of the surroundings

In another experiment with water as the liquid, the rate of cooling was investigated in the presence of the magnetic field, which was switched off for a few minutes and later re-established. The slopes of the cooling curves obtained in the magnetic field are essentially the same, and are greater than that of the curve obtained while the magnetic field was switched off. This further supports the finding that the cooling becomes faster in the presence of a high magnetic field. Experiments were also performed to study the effect of a magnetic field on the rate of heating for both the liquids, and it was found that the rate of heating is also faster in the presence of a magnetic field. Similar results were also obtained when the glass was replaced by a copper container.

From these experiments we conclude that the rate of cooling and also the rate of heating for these liquids increases in the presence of a high magnetic field.

The above results may possibly be explained on the basis of the change in specific heat of the liquid in a magnetic field or they may be due to a change in its absorptivity (or emissivity) in the presence of a magnetic field. In the case of mercury, however, there should be some additional effect due to the inhibition of convection in the magnetic field.

The nature of the variation of the rate of cooling of liquids subjected to different magnetic fields will be the subject of future investigation.

We are grateful to Dr. K. G. Ramanathan, of the National Physical Laboratory, New Delhi, for his

co-operation in allowing us to use the Laboratory's electromagnet.

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CHEMISTRY

Effect of Ultrasonic Insonation on the Carbon Tetrachloride–Water–Dissolved Helium System

It was the purpose of this study to obtain more evidence regarding the effect of ultrasonics on chemical reactions, and to determine the relationship between the reaction yield and solubility of dissolved gas in the system. Griffing[1] found that gases of different solubilities have different thresholds of cavitation; but no attempt was made to ascertain the effect of varying the solubility of a particular gas.

The system studied was the carbon tetrachloride–water–dissolved helium system. In order to ensure that the helium was the gas responsible for any reaction that might be observed, the water used was purified and degassed. Carbon tetrachloride was added and the mixture was vigorously agitated. Helium was then bubbled through, and after settling the substrate consisted of two distinct phases. The phase rich in carbon tetrachloride was used for the experiments. The carbon tetrachloride–water–dissolved helium mixture was then transferred (in a helium atmosphere) to a specially designed reaction vessel, one side of which was covered with a polyester film of high sound permeability. This served as a sound window.

The reaction mixture was exposed to 1,000 kc./s. sound energy at a power output of 7·20 watts/sq. cm. The sound was transmitted through a plain tap water medium from crystal to vessel. This water was circulated and served also as a constant-temperature bath. The distance between the crystal and reaction vessel surface was maintained the same for each run. This assured uniform sound absorbance by the sample. The samples after insonation were analysed for chlorine by addition of potassium iodide solution and the titration of the liberated iodine with sodium thiosulphate solution. Starch was used as indicator. It was found that the *p*H change brought about by the reaction was sufficient to supply the acid medium need for the above analysis.

Samples which were insonated immediately after they were transferred from the preparation apparatus to the reaction vessel gave erratic results. Samples which were transferred and allowed to stand 12 hr. before insonation gave much greater reproducibility. Griffing[1] observed a similar phenomenon. Different final concentrations of chlorine, due to insonation, were observed for vessels which were completely filled and those which were partially filled with reaction mixture (the vapour space being filled with helium). In addition to chlorine, it was thought that trace amounts of hexachloroethane could be detected.

The formation of chlorine and hexachloroethane from the reaction mixture, in the absence of oxygen, indicates a free-radical mechanism. This could well be due to the break-up of the water molecule as a result of local heating due to the adiabatic compression of the dissolved gas, as Griffing has suggested[2].

The different yields observed for partially filled and completely filled vessels can be ascribed to the variation in gas solubility with pressure. Ultrasonic insonation is a degassing process. It is obvious that the residual pressure in a vessel which was originally filled is much greater than the pressure in a vessel which was partially filled. According to Griffing's theory with regard to the effect of solubility on cavitation[1], the yield of chlorine would be greater at low pressures and lower at high pressures. The data show that the yield for filled vessels was lower than that for partially filled vessels. This supports the solubility theory of Griffing, using only a single gas. This eliminates any doubt which might have arisen due to the varying of solubility with different gases and thereby changing other physical properties as well.

The cause of the erratic results after sample transfer was probably nucleation—the formation of centres of gas bubble agglomeration. This would occur in random fashion for each run, thereby introducing a lack of similarity in sample preparation.

I would like to acknowledge the help of Dr. O. L. Updike during the course of the work, which was carried out at the University of Virginia, Charlottesville, Virginia.

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[1] Griffing, Sette, Sullivan and Majowicz, "Experiments on Cavitation and Related Reactions" (Catholic University, Washington, D.C.) (unpublished).
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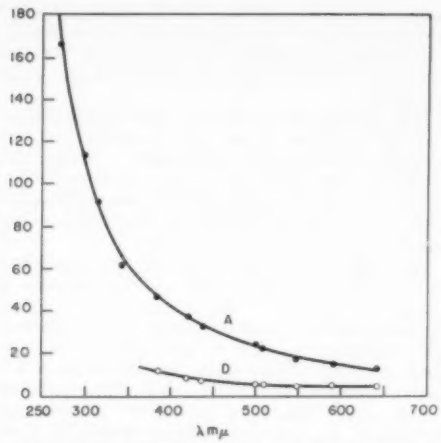
Optical Rotatory Dispersion Studies on Partially Methylated Cellulose

Rotatory dispersion studies have been conducted on a large number of proteins and polypeptides[1–3]. We wish to report some preliminary studies on the rotatory dispersion of polysaccharides. The material used for this work was 'Methocel' (registered trade mark of the Dow Chemical Company, Midland, Michigan) supplied by the Dow Chemical Co. 'Methocel' is a partially methylated cellulose which has the characteristic of being more soluble in cold water than in hot water. This investigation was carried out on material with an intrinsic viscosity of 1·6 (corresponding roughly to a molecular weight of 35,000) and a methyoxyl analysis of 29·5 per cent (1·8 OCH_3 groups/anhydroglucose unit). The method of solubilizing this particular polysaccharide consisted in dispersing the material in boiling water and allowing it to cool to room tem-



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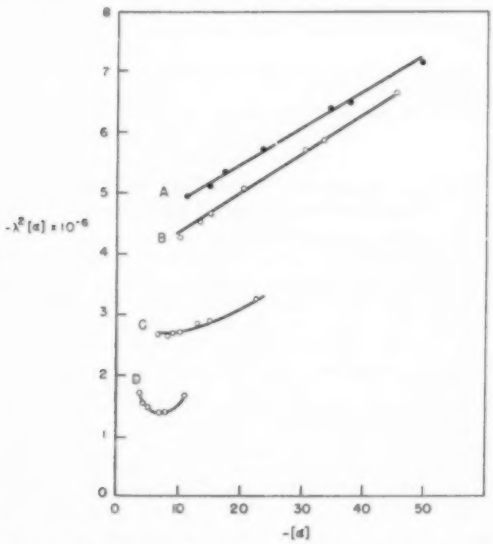

Fig. 2. Modified Drude plots of the dispersion data in Fig. 1. See text for description of curves *A*, *B*, *C* and *D*

The dispersion data were plotted by the method of Yang and Doty[2] as given in the following equation:

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(λ denotes wave-length in mμ, [α] specific rotation, λ_c the dispersion constant and K the intercept).

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Deuterium–Hydrogen Exchange between Water and Macromolecules: Accessibility of Cellulose

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			Brunauer–Emmett–Teller	Harkins–Jura	
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Deuterium loading was obtained by keeping the celluloses in contact with heavy-water vapour (99·7 per cent) for several hours, and then evacuating to constant weight. After four to six loadings, there was no further increase in the evacuated dried weights. The process was completely reversed by ordinary water vapour, so that there were no 'resistant' deuteroxyl groups produced[8]. The percentage gains in weight along with other results for the same substances[7,9] are given in Table 1.

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A Proposal for the Nomenclature of Free Radicals

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The naming of di-radicals would follow the usual practice for double bonds; for example, $\dot{C}H_2-CH=\dot{C}H$ would be called propenyl 1,3 di-el and $\dot{C}H=C=\dot{C}H$ propadienyl 1,3 di-el.

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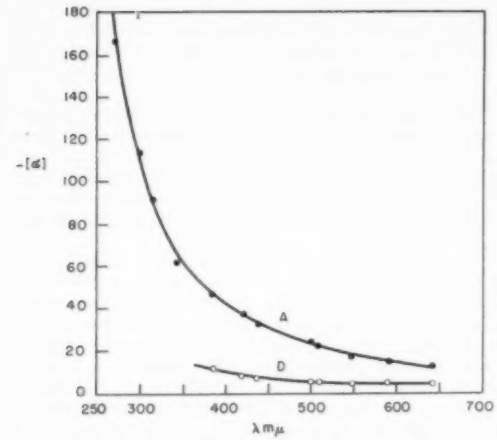

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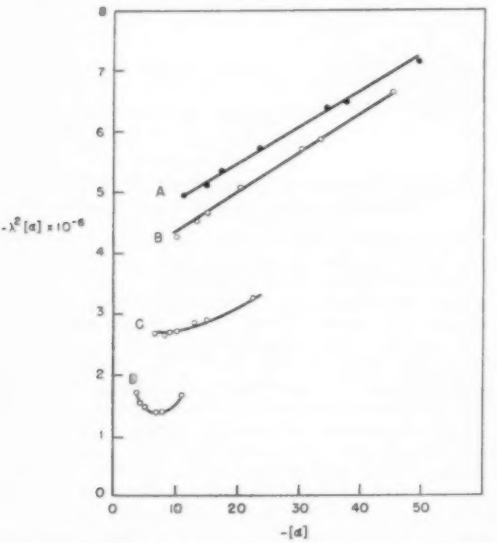

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BIOCHEMISTRY

Enzymatic Cycle of Inorganic Nitrogen in Animal Tissues

It has previously been demonstrated that oximino compounds are widely distributed in organisms[1], and that two enzymes, named oximase[2] and transoximase[3], which participate in oxime metabolism, exist in some animals. In pursuing the behaviour of hydroxylamine in living cells, we have now established that there is an enzymatic cycle between ammonia and nitrate.

An example of the procedure of preparing the enzyme solution was as follows: 30 gm. of hen liver were homogenized with 90 ml. of 0·1 *M* phosphate buffer of *p*H 7·5, and after 30 min. at 35° C., the homogenate was centrifuged at 3,000 r.p.m. for 10 min. at 0° C. The supernatant was treated with acetone at −10° C., and the precipitate which was produced at 33–55 per cent concentration of acetone was gathered by centrifuging at 4,000 r.p.m. for 7 min. 1 gm. of this precipitate was dissolved in 20 ml. of water, dialysed against running water for 3 hr. and centrifuged at 3,000 r.p.m. for 7 min. The supernatant was used as enzyme solution; it contains about 0·2 mgm. protein nitrogen in 1 ml. As the enzyme is labile, the solution must be prepared daily from fresh liver.

To measure the reducing action of enzyme preparation, 1 ml. of 0·001 *M* substrate was mixed with 1 ml. of enzyme solution, 1 ml. of 0·013 per cent reduced diphosphopyridine nucleotide solution, 0·5 ml. of 0·005 per cent flavin adenine dinucleotide solution and 0·5 ml. of 0·1 *M* phosphate buffer of *p*H 7·5. A mixture containing no substrate served as control. The reaction temperature was 30° C. and the time 20 min. The enzymatic activity was determined spectrophotometrically and expressed in decrease (−) of absorption (−log *T*) at 340 mμ. Some of the results obtained are given in Table 1.

Nitrate, nitrite, hyponitrite and hydroxylamine are thus reduced by an enzyme system from hen liver. It was further found that the enzymes manifest the highest activity at *p*H 7·7 and 30° C., and that they are completely destroyed by heating at 50° C. for 5 min. Reduced triphosphopyridine nucleotide and flavin mononucleotide do not act as coenzymes for the reduction by liver enzymes.

Recently, hyponitrite reductase was demonstrated in *Neurospora* by Medina and Nicholas[4], and hydroxylamine reductase was extracted from a halotolerant *Micrococcus* by Taniguchi, Sato and Egami[5] as well as from *Neurospora* by Zucker and Nason[6]. It remains to be seen whether the enzyme system of hen liver contains the same reductases as those found in microbes.

For estimating the dehydrogenating action of liver enzyme system, the freshly prepared enzyme solution was heated beforehand at 40° C. for 5 min., because the reductases are more easily inactivated by heating than the dehydrogenases. The reaction mixture consisted of 1 ml. of 0·001 *M* substrate, 1 ml. of enzyme solution, 1 ml. of 0·013 per cent diphosphopyridine nucleotide solution and 1 ml. of 0·05 *M* *tris*-buffer of *p*H 7·5. The control contained no substrate. The increase (+) of −log *T* at 340 mμ was determined after a 20 min. incubation at 30° C. Some of the results are given in Table 2.

Table 1. Reducing Action of Liver Enzyme System

Substrate :	$NaNO_2$	$NaNO_3$	$Na_2N_2O_2$	NH_2OH	$(NH_4)_2SO_4$	Without substrate
−log *T*	−0·105	−0·143	−0·124	−0·122	−0·024	−0·022

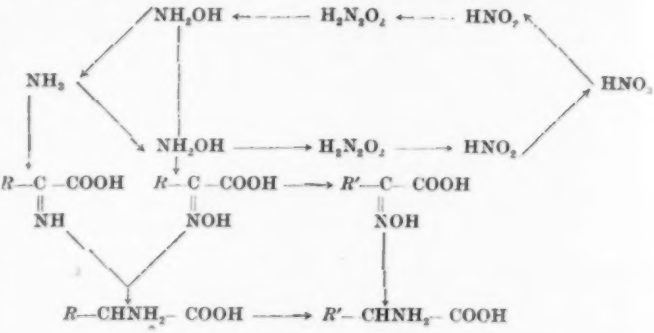
Table 2. Dehydrogenating Action of Liver Enzyme System

Substrate :	$(NH_4)_2SO_4$	NH_2OH	$Na_2N_2O_2$	$NaNO_2$	$NaNO_3$	Without substrate
−log *T*	+0·120	+0·095	+0·138	+0·084	+0·021	+0·015

Ammonia, hydroxylamine, hyponitrite and nitrite are thus dehydrogenated by the liver enzyme preparation. The dehydrogenation, however, does not proceed in a mixture containing phosphate buffer. It was further observed that the enzymes display the highest activity at *p*H 7·3–7·5 and 30° C., and that they are destroyed by heating at 60° C. for 5 min. Triphosphopyridine nucleotide, flavin adenine dinucleotide and flavin mononucleotide show no coenzymatic action for the dehydrogenation.

Klausmeier and Bard[7] reported recently that an ammonium dehydrogenase is present in *Bacillus subtilis*. However, both tri- and di-phosphopyridine nucleotide act as coenzymes for the bacterial dehydrogenase and ammonium sulphate does not serve as its substrate.

The enzyme solution was also prepared from silkworms, and its reducing and dehydrogenating actions were studied in the same way. Similar results were obtained.



Scheme 1. Inorganic nitrogen cycle and amino-acid formation

The combination of inorganic nitrogen cycle and amino-acid formation, including the transoximase–oximase reaction, can thus be formulated as in Scheme 1.

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Cysteine-S-sulphonate as an Intermediate in Microbial Synthesis of Cysteine

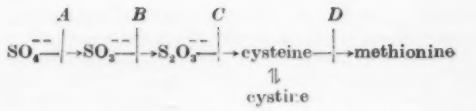
THE synthesis of cysteine in micro-organisms from inorganic sulphur sources has been studied for some time, and the available evidence suggests that sulphate sulphur is reduced to the thiosulphate or sulphide state prior to its introduction into the carbon chain of cysteine[1-3], although some investigators have postulated that the formation of sulphur–carbon linkage occurs at the sulphite stage[4]. Working with mutant strains of *Aspergillus nidulans*, Hockenhull[2] suggested that thiosulphate is the form in which sulphate sulphur combines with the carbon skeleton. Based on the observations that cysteine-S-sulphonate supported good growth of all the thiosulphate-requiring mutants tested and that growth of these strains on thiosulphate was enhanced by the addition of serine, he concluded that thiosulphate first reacts with serine to form cysteine-S-sulphonate as an intermediate and cysteine is formed from the latter by hydrolysis. The same possibility was also postulated by Shepherd[4], although the involvement of thiosulphate in the main route of sulphate assimilation was denied by this investigator. Schlossmann and Lynen[5], on the other hand, reported the occurrence in yeast of a pyridoxal phosphate-dependent enzyme catalysing the condensation of hydrogen sulphide with serine to form cysteine. The present communication describes more convincing evidence in favour of the cysteine-S-sulphonate pathway in *A. nidulans*.

Table 1. GROWTH OF MUTANTS ON VARIOUS SULPHUR SOURCES

Group	No. of mutants	Sulphur source added					
		Na_2SO_3	Na_2SO_4*	$Na_2S_2O_3$*	Cysteine*	Cystine	Methionine
Wild type	—	+	+	+	+	+	+
A	1	−	+	+	+	+	+
B	1	−	−	+	+	+	+
C	6	−	−	−	+	+	+
D	6	−	−	−	−	−	+

+, Growth; −, no growth (after seven days). The basal medium was supplemented with 100 μgm. per ml. of sulphur compound. All amino-acids used were L-isomers. Incubated at 32° C.
* Sterilized by Seitz filtration.

Mutant strains of *A. nidulans* (I.F.O. Collection No. 5713) requiring methionine for growth were isolated by Fries's filtration technique[6] from conidiospore suspensions exposed to ultra-violet irradiation, and 14 stable mutants were obtained from about 700 isolates. The responses of these strains as well as that of the parent wild-type strain are summarized in Table 1. The basal sulphur-deficient medium used in these experiments contained per litre 30 gm. glucose, 3 gm. sodium nitrate, 1 gm. dipotassium hydrogen phosphate, 0·5 gm. potassium dihydrogen phosphate, 0·2 gm. magnesium chloride, 1 ml. of trace element solution, and 17 gm. of agar (*p*H 6·4). (The trace element solution contained in mgm. per litre: $FeCl_2.6H_2O$, 1,000; $CuCl_2.2H_2O$, 300; $MnCl_2.4H_2O$, 72; $(NH_4)_6Mo_7O_{24}.4H_2O$, 40; $ZnCl_2$, 4,200.) All the growth tests were carried out at least twice. The results obtained indicate that the mutants can be classified into four groups, namely, *A*, *B*, *C* and *D*, according to their nutritional requirements. It appears that the simplest explanation for these findings as well as those reported in previous work[2,3] may be provided by the following scheme of sulphate assimilation:



where the site of block in each group of mutants is indicated by *A*, *B*, etc.

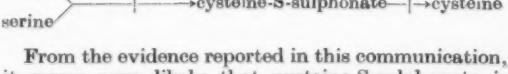
Having assumed the correctness of this pathway, the mechanism of the incorporation of thiosulphate into cysteine was then studied using the 6 strains belonging to the *C* group in which this very reaction is genetically blocked. If this step of assimilation actually involves cysteine-S-sulphonate as an intermediate, it is expected that this step is controlled by at least two genes. This possibility was examined by subjecting the 6 strains to the heterokaryosis test on the basal sulphur-deficient medium supplemented with 200 μgm. of sodium sulphate and 5 μgm. of L-cystine (for heterokaryosis see ref. 7). It was thus found and confirmed by repeated experiments that one strain (designated as 793) can form heterokaryons with any of the other 5 strains (designated as 562, 611, 649, 721 and 831). No signs of heterokaryosis could, however, be detected in any pair of the latter 5 strains, indicating their genetical identity.

Table 2. UTILIZATION OF CYSTEINE-S-SULPHONATE BY MUTANTS OF THE *C* GROUP

Strain	Wild type	793	562	611	649	721	831
Growth (mgm. dry wt./15 ml.)	57·8	3·0	49·5	37·3	19·0*	39·3	40·8

Average of four experiments. The basal liquid medium was supplemented with 100 μgm. of Seitz-sterilized sodium cysteine-S-sulphonate per ml. Incubated at 33° C. for ten days without shaking.
* This strain showed rather poor growth even in a methionine medium.

Since strain 793 was thus genetically differentiated from the other 5 strains of the *C* group, it seemed of considerable interest to study the ability of these mutants to utilize cysteine-S-sulphonate for growth. The growth experiments were therefore conducted in a liquid medium containing 100 μgm. of sodium cysteine-S-sulphonate (prepared by a modification of the method of Kolthoff and Stricks[8]) per ml. as the sole source of sulphur. The *p*H of the medium was raised to 7·5 to minimize the spontaneous decomposition of the sulphur compound. As can be seen from Table 2, it was clearly shown that strain 793 is quite unable to grow in this medium, whereas the same medium can support good growth of the other 5 strains as well as the wild-type strain. These results, together with those of Table 1, strongly suggest that strain 793 has a genetical block between cysteine-S-sulphonate and cysteine and the other 5 strains are deficient in the enzyme responsible for the condensation of thiosulphate with serine (or other 3-carbon compounds) to form the sulphonate. This situation will be understandable from the following scheme:



From the evidence reported in this communication, it seems very likely that cysteine-S-sulphonate is involved in the normal pathway of cysteine biosynthesis in *A. nidulans*. The occurrence of this pathway receives further support from the finding that the sulphonate can be detected polarographically in a

reaction mixture containing a cell-free yeast extract, thiosulphate, serine and pyridoxal phosphate[9].

We wish to thank Mr. S. Baek for the synthesis of sodium cysteine-S-sulphonate.

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Shape of the Precipitate in Immunoelectrophoresis

In immunoelectrophoresis, according to Grabar and Williams[1], diffusion of the electrophoretically separated antigens towards the immune serum added in longitudinal basins parallel to the migration axis after the electrophoretic separation will result in the formation of precipitates in the gel, occupying varying positions between antigen and antibody diffusion centres as well as varying electrophoretic positions.

These precipitates generally appear as curved lines with their convexity towards the antibody diffusion centre, because of the immune serum diffusing in a straight line in a direction perpendicular to the electrophoretic axis, while the antigens diffuse from roughly circular diffusion centres in the agar. The point of the precipitate which is nearest to the antibody basin, the peak of the precipitate, is taken to indicate the average electrophoretic position of the electrophoretically separated component giving rise to this precipitate[2].

In some instances, precipitates are found which deviate from the half-moon-shaped symmetrical precipitates by presenting varying degrees of curvature or possessing different degrees of asymmetry. Also, precipitates may be found which have more than one peak. It has been found that these different shapes of the precipitates are remarkably constant for the same precipitating component, when sera from normal individuals are investigated under standard experimental conditions[3,4].

Grabar[5] demonstrated that the γ-globulin precipitate in normal human sera, which incidentally appears as an extended line rather than a curved half-moon-shaped precipitate, consisted of a "family of proteins", each molecule population with its own specific electrophoretic mobility although serologically identical.

In certain human sera, an extended precipitate is obtained in the α_2-globulin region, which therefore according to Grabar's findings might indicate the presence of two or more molecule populations being serologically identical, although presenting differences in electrophoretic mobilities. Also, at least two molecule populations with discrete electrophoretic positions are present, as indicated, by obtaining a precipitate with two peaks if the separation is prolonged[6].

In other normal human sera either the fast-moving molecule population or the slowly moving molecule

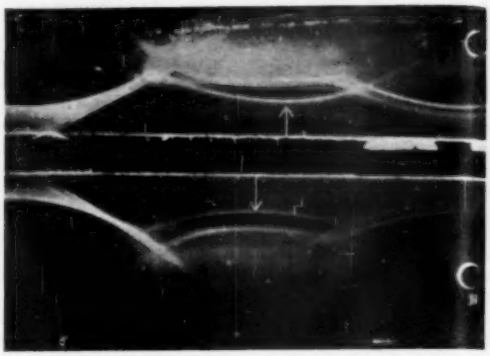

Fig. 1. Immunoelectrophoresis of mixture of sera belonging to *G.c.*1 : 0·1 ml. and *G.c.*2 : 0·4 ml. (upper basin) and mixture of the same sera in equal amounts (lower basin). To the far left the albumin-precipitate is shown and to the right the starting point and the transferrin-precipitate. The group-specific components are indicated by arrows

population is present. Mixture of sera containing the fast-moving and slowly moving precipitating components in equal amounts will result in the immunoelectrophoretic appearance of a precipitate which is extended or presents two peaks, depending on the separation time and which is indistinguishable from the same precipitate found in certain other normal sera[6]. Also, evidence for the genetic inheritance of these group-specific components has been found[7].

If sera belonging to the fast type (*G.c.*1) and the slow type (*G.c.*2) are mixed in unequal amounts, for example, in the proportions of 1 : 4, an asymmetric precipitate will occur where the peak of the precipitate is displaced from the midpoint towards the cathode (Fig. 1). In a similar way an asymmetric precipitate with its peak displaced towards the anode will be obtained if the fast-moving precipitating component is quantitatively predominant in the mixture.

This seems to demonstrate that the electrophoretic positions of the precipitates can be changed by quantitative factors. This change of electrophoretic position is even more pronounced if sera belonging to different groups are mixed in the proportions 1 : 10. If the slowly moving precipitating compon-

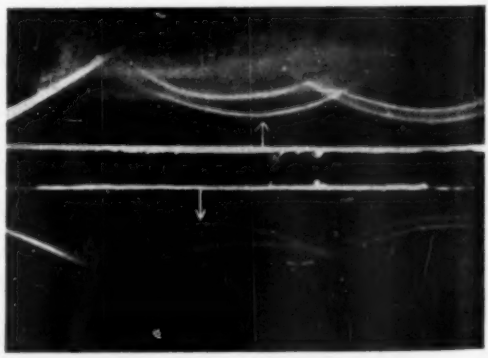

Fig. 2. Immunoelectrophoresis of mixture of sera belonging to *G.c.*1 : 0·01 ml. and *G.c.*2 : 0·1 ml. (upper basin) and mixture of the same sera in reversed proportions (lower basin). To the far left the albumin-precipitate is shown and to the right the transferrin-precipitate. Differences in electrophoretic positions of group-specific components are indicated by arrows

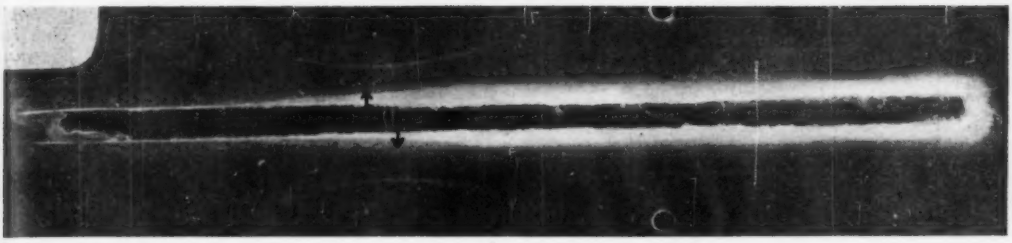

Fig. 3. Immunoelectrophoresis of mixture of sera belonging to *Hp* 1–1 and 2–2 in the same amounts (upper basin) as compared to haptoglobin-precipitate of a serum belonging to *Hp* 2–1 (lower basin). By means of an absorption technique only the haptoglobin-precipitates appear in the α_2-globulin region

ent is quantitatively predominant in the mixture, immunoelectrophoresis will demonstrate a precipitating component occupying an electrophoretic position more towards the cathode than if the fast-moving precipitating component is quantitatively predominant in the mixture (Fig. 2). None the less, the differences are not as pronounced as when the precipitates of unmixed sera belonging to these two different groups are compared.

With the haptoglobins, results of a similar nature have been obtained. These proteins have been demonstrated to present a considerable degree of 'microheterogeneity' in starch-gel electrophoresis. Three main groups can, however, be readily differentiated in starch-gel electrophoresis[5], agar-gel electrophoresis[3] and immunoelectrophoresis[6] although immunologically identical[10]. Mixture of sera belonging to *Hp* 1–1 and 2–2 in equal amounts resulted in a single line in immunoelectrophoresis, not occupying the same electrophoretic position, however, as the normally occurring heterozygote *Hp* 2–1, but being more displaced towards the anode (Fig. 3). If the mixtures consist of unequal amounts of these homozygotic haptoglobin groups, varying degrees of asymmetry and displacement of the precipitates will be observed (Fig. 4).

Therefore, it seems as if the shape of the precipitates might serve as a valuable method of characterization of certain precipitating components and also that information will be obtained as to degree of 'microheterogeneity' of proteins, being at least from the serological point of view characterized as homogeneous and where also proof of heterogeneity is not obtained by conventional electrophoretic methods. Also, the electrophoretic position of precipitates might be regarded as a reflexion of the average distribution of serologically identical molecule populations in the electrophoretic field, whereas the shape of the precipitates might indicate the quantitative distribution or scatter of molecule populations in the electrophoretic field from a serological point of view.

However, also other factors might affect the shape of the precipitate, for example, tailing phenomena and ratio of molecular weights between the reactants participating in the precipitate formation[11]. Finally, it must be pointed out that a serological identity-reaction might result between components which are not identical when other immune sera are used to develop the precipitates. This has been observed for some precipitating components in human sera and it seems as if different immune sera might be able to show varying degrees of reactivity with different antigenic components. In these instances an extended precipitate might be expected to show a resolution into two or more normally arc-shaped precipitates with suitable immune sera[12]. In the above-quoted examples, however, no evidence has as yet been obtained for serological differences within the haptoglobins or the other group-specific system although investigated against about 40 different immune sera prepared on rabbits and one prepared on horse.

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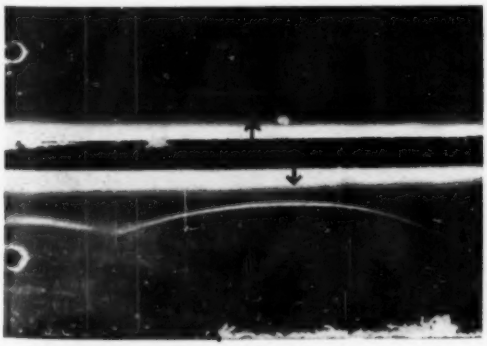

Fig. 4. Immunoelectrophoresis of mixture of *Hp* 1–1 : 0·4 ml., and *Hp* 2–2 : 0·1 ml. (upper basin) and in reversed proportions (lower basin). By means of absorption, only the haptoglobin-precipitates are visible in the α_2-globulin region

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An Anthocyanin-decolorizing System in Sour Cherries

THE anthocyanins of many fruits contribute to their attractiveness and appeal. In the course of a general study of the anthocyanins we have measured the accumulation of anthocyanins together with the activity of an anthocyanin-decolorizing system in Montmorency and English Morello sour cherries[1]. Anthocyanin-decolorizing systems have previously

Table 1. Anthocyanin-decolorizing Ability and Anthocyanin Accumulation in Maturing Sour Cherries

Date picked 1959	Montmorency		English Morello	
	Decolorizing ability (units/gm.)	Anthocyanin* accumulation (*O.D.* at 510 mμ)	Decolorizing ability (units/gm.)	Anthocyanin* accumulation (*O.D.* at 510 mμ)
May 29	2,740	0·515	3,200	0·875
June 9	4,950	—	5,580	—
June 19	4,680	—	6,080	—
July 2	3,790	1·80	—	—
July 9	—	—	6,950	11·0
July 15	4,075	21·9	5,560	65·4
July 24	4,220	24·2	5,310	114·0
Aug. 4	2,760	16·4	3,240	101·0
Aug. 19	3,000	14·4	2,530	113·0

* Calculated as the *O.D.* of extract from 1 gm. dry weight of cherries dissolved in 1 ml. of solution.

been reported in leaves[2–4] and these, in common with the system found in cherries, apparently involve the action of an oxidizing enzyme.

The activity of the anthocyanin-decolorizing system was assayed as described by Wagenknecht *et al.*[5] using cyanidin-3-rhamnoglucoside[6] as the substrate. The enzyme was supplied as 0·5 ml. of a homogenate containing 20 per cent wet weight of pitted cherries. The results are expressed as units per gm. dry weight of cherry tissue. Anthocyanin accumulation in the cherries was measured by heating pitted cherries in McIlvaine buffer[7], *p*H 3·4, to 71·1° C., homogenizing in a blendor, filtering and diluting the extract with buffer as needed for measurement of absorption at 510 mμ. Results were converted to optical density per ml. of extract per gm. dry weight. Cherries were harvested from late May until the middle of August.

The ability of homogenates of Montmorency and English Morello cherries to catalyse the decolorization of cyanidin-3-rhamnoglucoside increased rapidly with the age of the cherries up to a period of maximal activity and then decreased as the cherries became more mature (Table 1). The peak activity of the Montmorency sour cherries occurred about a month earlier than that found with the slower maturing English Morello variety. With both varieties the highest anthocyanin-decolorizing ability was detected when the cherries had accumulated relatively little red pigment.

The accumulation of anthocyanin (Table 1) continued throughout the experimental period in the case of the English Morello cherry, but accumulation of pigment in the Montmorency cherry reached a maximum several weeks before the experiment was ended.

In order to obtain maximum decolorization by the cherry homogenates it is necessary to add catechol to the reaction mixture[5]. In the course of the investigation reported here it was found that this was true throughout the sampling period. There was a difference between cherry varieties with regard to the amount of activity retained when catechol was not included in the reaction mixture. Upon the omission of catechol English Morello homogenates retained 33 per cent of their decolorizing activity while Montmorency homogenates retained only 20 per cent of their activity found when catechol was included. This may be due to the two varieties having different amounts of substances, such as polyphenols, which might play a part similar to that of catechol in promoting the decolorization of anthocyanin.

Relatively greater anthocyanin-decolorizing ability was found in those tissues which accumulated the greatest amount of anthocyanin. For example, the more heavily pigmented English Morello cherries were more active than the Montmorency cherries. In both varieties the activity of the skins was greater than that of the less highly pigmented flesh. It is not yet known if this relationship has any significance with respect to anthocyanin synthesis.

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Polysaccharide Composition of Leaves

Plum leaves (*Prunus domestica*) contain sufficient amounts of starch, cellulose, hemicellulose, and pectic substances to recommend them for studies of the biosynthesis and possible inter-relationships of these leaf polysaccharides and their monosaccharide precursors[1]. Experiments involving photosynthesis by plum leaves in carbon dioxide labelled with carbon-14 and subsequent fractionation of the leaf polysaccharides revealed marked differences, both in the extent to which carbon-14 was incorporated into the different monosaccharides of each fraction, and also in the specific activities of each monosaccharide from different fractions[2,3]. Further investigations could be facilitated by using leaves relatively richer than plum leaves in any one polysaccharide constituent. There is little information on this point, consequently a diversity of leaves has been examined for polysaccharides (Table 1).

The leaves of wheat and gooseberry were picked in July, those of all other species were gathered during the afternoons in August and early September, and immediately immersed in ethanol. After exhaustive extraction with methanol, the leaf residues were hydrolysed (*N* sulphuric acid at 100° for 16 hr.) and, after neutralization (barium carbonate), the products examined on paper chromatograms in two solvent systems (ethyl acetate/acetic acid/water, 9 : 2 : 2 : butan-1-ol/pyridine/water, 10 : 3 : 3 parts v/v)[1]. The relative amounts of the monosaccharides present in each hydrolysate were estimated visually from the size and intensity of the spots revealed on chromatograms with ammoniacal silver nitrate and *p*-anisidine hydrochloride[5]. The presence of small amounts of acid-stable aldobiouronic acids was noted in some of the hydrolysates. Neither glucurono-1 → 4-lactone nor hexuronic acid monomethyl ether was detected, thus suggesting that galacturonic acid was the major acid component. No attempt was made to study seasonal or diurnal variations in composition, or to determine the amount of fructan, if any, or cellulose present in the leaves.

For the species so far investigated, the leaf polysaccharides were composed of a variety of monosac-

Table 1. Relative Amounts* of Monosaccharides detected in Leaf Polysaccharide Hydrolysates

Family	Species	Galactose	Glucose	Mannose	Arabinose	Xylose	Rhamnose	Hexuronic acid
Taxaceae	*Taxus baccata* (yew)	+	+	t	++	t	–	+
Pinaceae	*Picea abies* (Norway spruce)	t	++	t	+	t	–	(+)
	Pinus sylvestris (Scots pine)	+(+)	++	++	++	+	t	(+)
	Pseudotsuga taxifolia (Douglas fir)	+	++	t	++	+	–	+
	Cedrus atlantica (Mt. Atlas cedar)	t	+++	t	+	t	–	+
Taxodiaceae	*Sequoia sempervirens* (redwood)	+	+	t	+	+	–	(+)
Cupressaceae	*Cupressus lawsoniana* (Lawson cypress)	+	+++	t	++	+	–	+
	Thuja orientalis (Chinese arbor vitae)	+	t	t	++	t	–	+
Fagaceae	*Fagus sylvatica* (common beech)	+	+	t	+	+++	–	(+)
	Quercus pedunculata (English oak)	+	+	–	++	++	–	+
Crassulaceae	*Sedum acre* (wall pepper)	+	++	–	+	t	–	+
Rosaceae	*Pyrus malus* (apple, var. Bramley seedling)	+	++	t	++	+(+)	t	+
	Pyrus communis (pear, var. Conference)	+	++	t	++	+	t	(+)
	Prunus domestica (plum, var. Victoria)	+	++	t	++	+	(+)	+
	Rubus fruticosus (cultivated bramble)	+	++	—	+	+	t	+
	Sorbus aria (white beam-tree)	+(+)	+	—	++	++	(+)	+
Saxifragaceae	*Ribes uva-crispa* (gooseberry)	+	++	t	+	(+)	(+)	++
Leguminosae	*Vicia faba* (broad bean)	+	+++	–	+	(+)	t	(+)
	Phaseolus vulgaris (runner bean)	+	+++	t	(+)	(+)	t	+
	Trifolium pratense (clover)	+(+)	++	–	++	+	t	+
Geraniaceae	*Pelargonium hortorum* (cultivated 'geranium')	+	+++	t	(+)	(+)	t	t
Euphorbiaceae	*Ricinus communis* (castor oil plant)	++	+++	–	+	+	t	+(+)
Aquifoliaceae	*Ilex aquifolium* (holly)	++	+	–	+++	+++	t	++
Hippo-castanaceae	*Aesculus hippocastanum* (horse chestnut)	+	+++	–	+	+	t	+
Araliaceae	*Hedera helix* (ivy)	+	++	t	+	+	t	+
Labiatae	*Mentha gentilis* (cultivated mint)	+	+++	t	(+)	(+)	t	+
	Salvia officinalis (cultivated sage)	+(+)	+++	t	++	+	t	+
Gramineae	*Triticum aestivum* Svenno (wheat)	t	+	–	+	+++	–	t

* In order of increasing magnitude the symbols are: – (not detected), t (trace), (+), +, +(+), ++, +++.

charide units, galactose, glucose, arabinose, xylose and hexuronic acid being common to them all and mannose and rhamnose frequently occurring in trace amounts. Glucose in the hydrolysates probably arose from starch which, as expected, was predominant in the leaves of many species, including cedar, bean, mint, and *Pelargonium*. Only in Scots pine needles was mannose found in more than trace amounts. Plum leaf polysaccharides were typical of the Rosaceae species examined, with roughly similar amounts of hexose and pentose components. Of particular interest for biosynthetic studies, however, were those leaves rich in pentans. For example, arabinose and xylose together formed a large part of oak and holly leaf polysaccharides, the latter apparently consisting almost entirely of xylan and the pectic substances, while xylan was a major component of wheat and beech leaves, and in yew and *Thuja orientalis* the araban component was predominant.

Wheat plants have already been used successfully to investigate xylan and cellulose biosynthesis[4], and the results presented here indicate the utility of other species for studies of polysaccharide metabolism. In this respect further studies are in progress.

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PHYSIOLOGY

Effect of Cytotoxic Agents on Antibody Production

It has long been known that antibody-production may be inhibited by certain cytotoxic agents, for example, X-radiation, benzene and mustard gas[1]. More recently, the number of agents reported to have this effect has been extended to include cortisone[2], nitrogen mustards[3], colchicine[4], deoxypyridoxine[5], 8-azaguanine[6], various carcinogens[7], β-3-thienylalanine[8], 6-mercaptopurine[9] and amethopterin[10]. Many of these are potent anti-tumour compounds.

The immune response produced by the uptake and catabolism of antigen may be divided into: (1) the induction phase, usually lasting some days, during which antibody is not produced; (2) the production phase, in which antibody protein is synthesized and released. X-rays and cortisone inhibit the immune response mainly by acting on the induction phase, although they may also affect phagocytosis. If exposure to these agents is delayed until antibody-synthesis is proceeding, their effect is diminished or absent[11]. There is evidence that mustards[12] and 6-mercaptopurine[9] behave similarly. Since these agents strongly inhibit nucleic acid synthesis, and particularly synthesis of deoxyribonucleic acid, and have much less effect on protein synthesis, these observations are relevant to the widely held belief that the induction phase is concerned with antigen-induced changes in nucleic acid metabolism[13].

With other agents inhibiting the immune response, most work has involved repeated injections of agent or antigen or both, so that the critical time of the agent's action is obscured. Such substances as β-3-thienylalanine and deoxypyridoxine, which require special dietary conditions for their action, probably inhibit the immune response largely by interfering with the production of protein, and their anti-tumour action also is probably mainly nutritional.

Table 1

Compound	Dose (mgm./kgm.)	Deaths	Titres (log_4)		
			Test	Control	Difference
Group *A*					
Amethopterin	20	2/5	9	7	+ 2
8-Azaguanine	1,500	3/5	8	7	+ 1
Chlorambucil	35	0/5	8	10	− 2
Daraprim	700	3/5	4	7	− 3
6-Mercaptopurine	150	1/5	3	7	− 4
Methyl acetamide	2,750	1/5	9	8	+ 1
Methyl *bis*(β-chloroethyl)amine	2·4	2/5	8	7	+ 1
Methyl formamide	5,000	1/5	8	8	0
Ribonuclease	2,500	0/5	6	8	− 2
Stilbœstrol	1,000	3/5	7	8	− 1
Triethylene melamine	2	2/5	5	8	− 3
Urethane	1,250	1/5	9	8	+ 1
Group *B*					
8-Azaxanthine	3,000	3/5	7	8	− 1
Benzimidazole	600	3/5	9	8	+ 1
Boric acid	2,500	3/5	9	8	+ 1
Caffeine	200	1/5	8	8	0
Deoxypyridoxine	275	1/5	8	8	0
D-Glucosamine	8,000	3/5	7	8	− 1
Methyl carbamate	1,000	1/5	6	7	− 1
Ovalbumin (hen)	2,500	0/5	7	7	0
Potassium cyanide	7·5	1/5	8	8	0
Serum albumin (bovine)	2,500	0/5	8	7	+ 1
Streptomycin sulphate	750	2/5	8	7	− 1
β-2-Thienylalanine	5,000	1/5	9	8	+ 1

It was thought that investigations along these lines might throw further light both on the initiation of the immune response and on the mode of action of anti-tumour compounds. A wide range of substances was therefore examined, by administering simultaneously single doses of substance and antigen, and titrating antibody 10 days after the injection. By this timing it was hoped to identify substances that interfere with the induction phase of the immune response and do not act simply by impairing protein synthesis.

Male *A2G* mice, weighing 17–25 gm., were given single subcutaneous injections of the compounds studied and, immediately afterwards, 0·2 ml. T.A.B. vaccine intraperitoneally. The compounds were dissolved or suspended in 0·85 per cent saline or, when appropriate, in arachis oil. Controls received 1 ml. saline or arachis oil. Ten days later serum from groups of 5 mice was pooled and titrated for *Salmonella typhi H*-agglutinins. Titres are expressed in Table 1 as logarithms to the base 2, and a reduction of 2 in titre was regarded as significant evidence of inhibition.

Each compound was tested at several dose-levels and, except for the proteins, which present difficulties in this respect, the range included doses that killed a proportion of the animals. Table 1 gives only the results obtained at the higher end of this range. Published toxicity-levels were only a rough guide to dosage, owing partly to concurrent administration of vaccine. Colchicine, for example, could not be tested satisfactorily; its *LD*50, normally 1·75 mgm./kgm. in these mice, was 0·35 mgm./kgm. when the vaccine was given simultaneously.

In Table 1 group *A* consists of compounds that are used clinically against human tumours, leukæmia or polycythæmia vera or that are undoubtedly effective against transplanted animal tumours without requiring special dietary conditions. Group *B* are not effective antitumour agents as judged by these criteria.

Of the 24 compounds, five significantly reduced antibody-production. All were in group *A*. Four of these are clinically useful in human malignant disease. The fifth, ribonuclease, is said to be active against various mouse tumours[14], but there has been no published report of its effect on human neoplasms. No compound ineffective against tumours ever caused significant impairment of antibody-production under the conditions of this experiment, although most of them were tested almost to the limit of tolerance.

Some of the group *A* compounds inhibited antibody-production in non-lethal doses, whereas group *B* included compounds that were ineffective in doses that killed most of the animals. It is therefore unlikely that the inhibition of the immune response was due to general toxicity. In fact, the doses were such that the same proportion of deaths occurred in both groups.

Ribonuclease appears of special interest, but, since it is itself an antigen, the possibility that it acts here by 'antigenic competition' cannot yet be excluded, although the other proteins tested failed to inhibit antibody production. Deoxyribonuclease, 1,000 mgm./kgm., had no effect on the antibody titre.

These preliminary results suggest that this experimental system may provide a useful adjunct to other methods for screening anti-tumour compounds.

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Influence of Insulin on the Incorporation of 2-^{14}C-Sodium Pyruvate into Glyceride Glycerol in Diabetic and Normal Baboons

Depancreatized baboons, deprived of insulin for 4 days, develop a lipæmia and a fatty liver, which may contain 34 per cent of fat when estimated on a wet-weight basis[1,2]. The lipæmia in diabetic rats has generally been attributed to mobilization of fat from the depots. In the baboon, the severity of the lipæmia and the degree of fat accumulation in the liver, particularly in young animals with poorly developed fat depots, suggested that the fat was not

mobilized only from the depots, and this warranted a re-investigation of the origin of the fat in the liver and in the plasma. In this communication it is proposed to report on the incorporation of 2-^{14}C sodium pyruvate into the glyceride glycerol in a normal baboon and in depancreatized baboons either receiving insulin or deprived of insulin.

Five animals were used in this study. General data on the animals are given in Table 1.

Table 1. Data on Experimental Baboons

Baboon	Age (yr.)	Weight (kgm.)	Sex	Treatment	Duration of pancreatectomy
440	± 6–7	14	F	Normal	—
422	± 6	12·7	F	Depancreatized on insulin	7 months
355	6	20	M	Depancreatized without insulin	2½ years
448	± 3	11	M	Depancreatized without insulin	2½ months
403	3½	8·1	M	Depancreatized without insulin	9 months

During the course of the experiment, baboons 355, 448 and 403 received no insulin for 96 hr. Each animal received a total of 500 μc. of 2-^{14}C sodium pyruvate (supplied by the Radiochemical Centre, Amersham) in three divided doses injected intravenously as a saline solution over a period of three days. 6 hr. after the last pyruvate injection blood was drawn for the isolation of serum lipids; at the same time, samples of liver and depot fat were removed and extracted for fats. The triglyceride fractions of the fats were separated by chromatography on silicic acid. The triglycerides were hydrolysed by boiling with alcoholic potassium hydroxide. After extraction of the fatty acids with ether, the glycerol was isolated from the aqueous fraction as glycerol tribenzoate. The substance was recrystallized from methanol and the purity of the material was checked by melting point determination. A further check on the purity of the material was obtained from the wet combustion to carbon dioxide according to the method of van Slyke, Plazin and Weisiger[3]. All samples were counted as barium carbonate with a proportional gas counter. Details of the method used for plating and counting will be published shortly.

The results of the incorporation of carbon-14 into the glyceride glycerol of serum, liver and depot fat in the five baboons are shown in Table 2. We have demonstrated that it is possible to calculate, with a reasonable degree of accuracy, the specific activity of the glycerol from the observed total activity of the unhydrolysed triglyceride fraction after deduction of the activity due to the fatty acids. As limited amounts of triglycerides were obtained from the serum of baboons 422 and 440, we have no information on the radioactivity incorporated into the glycerol in these cases. However, the evidence is very suggestive that the levels in the serum reflect those in the liver and we suspect that the radioactivity in the glyceride glycerol of the serum in these two baboons was low.

Table 2. Specific Activities of Glyceride Glycerol of Serum, Liver and Depot Fat (counts/min./*m*M) of Depancreatized Baboons after Administration of 2-^{14}C Sodium Pyruvate

Baboon No.	Treatment	Serum	Liver	Depot fat
355	Depancreatized without insulin	62,916*	73,268*	3,956*
			68,632	2,484
448	Depancreatized without insulin	—	99,544	13,064
403	Depancreatized without insulin	120,888*	111,504*	6,532
		113,449		
422	Depancreatized on insulin	—	0	0
440	Normal	—	0	0

* Calculated.

Attention is directed to the following experimental findings: (1) Carbon-14 is markedly incorporated into the glyceride glycerol of the liver and plasma and to a much lesser extent into the glycerol of the fat depots (Nos. 355, 448 and 403, Table 2). (2) The administration of insulin to the depancreatized baboon completely blocks the incorporation of carbon-14 into the glycerol of liver and depot fat (No. 422, Table 2). (3) Incorporation does not occur in the normal baboon (No. 440, Table 2). (4) The extent of the incorporation is the same in the glyceride glycerol in the serum and in the liver of depancreatized insulin-deprived baboons, as determined by experiment or by calculation (Nos. 355 and 403). (5) The extent of incorporation is from 30 (No. 355) to 200 times greater (No. 403) in the liver glycerol than in the fat depots.

These observations, taken in conjunction with others relating to the fatty acids (unpublished results), would seem to indicate that if fat is mobilized from the depots it is not mobilized as triglycerides of the depots but is hydrolysed before entering the circulation. Furthermore, the close correspondence between the glycerol in the liver and serum would suggest that the triglycerides of the serum are derived from the liver and not primarily from the depots. The blocking effect of insulin on the synthesis of glycerol from pyruvate or from metabolites derived from pyruvate is a noteworthy phenomenon.

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Action of Thyroxine Analogues on Frog Melanocytes

The melanocyte-stimulating hormones and caffeine darken frog melanocytes by causing dispersion of melanin granules throughout the cell. While the end effect of these two substances on melanocyte opacity is the same, there are several differences in the way melanocyte-stimulating hormone and caffeine bring about dispersion. Lightening of melanocytes is produced by aggregation of melanin granules towards the centre of the cell. We found that triiodothyronine was an effective lightening agent of frog skin previously darkened by melanocyte-stimulating hormone but had no action on caffeine-treated skin. Because of this result we decided to test thyroxine and triiodothyroacetic acid for lightening properties.

Skin from the legs and thighs of eight frogs (*Rana pipiens*) was mounted on rings[1] and arranged in four comparable groups as follows:

Group *A*	LLg1	LTh2	RTh3	RLg4	LLg5	LTh6	RTh7	RLg8
Group *B*	RLg1	LLg2	LTh3	RTh4	RLg5	LLg6	LTh7	RTh8
Group *C*	RTh1	RLg2	LLg3	LTh4	RTh5	RLg6	LLg7	LTh8
Group *D*	LTh1	RTh2	RLg3	LLg4	LTh5	RTh6	RLg7	LLg8

L, left; R, right; Lg, leg; Th, thigh.

Before any observations were made, all skin specimens were soaked in four changes of frog Ringer's solution for 2 hr. Each specimen was maintained in 20 ml. of Ringer's solution throughout the experiment. Changes in the skin were measured with a photoelectric reflexion meter[1]. The initial reading of each sample was taken as its individual base-line, and increase or decrease in reflectance was measured from this.

Two groups were treated with 7·5 units of melanocyte-stimulating hormone per 20 ml. Ringer's solution, and two groups were treated with 20 mgm. of caffeine per 20 ml. Ringer's solution. These quantities of melanocyte-stimulating hormone and caffeine produced approximately the same degree of darkening. The melanocyte-stimulating hormone had been made from bovine posterior pituitary powder[1].

Pronounced darkening occurred in all groups and was followed as a decrease in reflectance for 60 min. At this time sodium triiodothyronine was added to one group treated with melanocyte-stimulating hormone and to one group treated with caffeine. The final concentration of the hormone was 5 μgm. per ml. Changes in reflectance were followed for 50 min. more. As shown in Fig. 1, marked lightening occurred in the group treated with melanocyte-stimulating hormone following the addition of triiodothyronine. There was no change in the group treated with caffeine.

The experiment was repeated using thyroxine as the sodium pentahydrate salt and triiodothyroacetic acid as the diethanolamine salt. Again marked lightening occurred in the groups previously treated with melanocyte-stimulating hormone but not with those treated with caffeine.

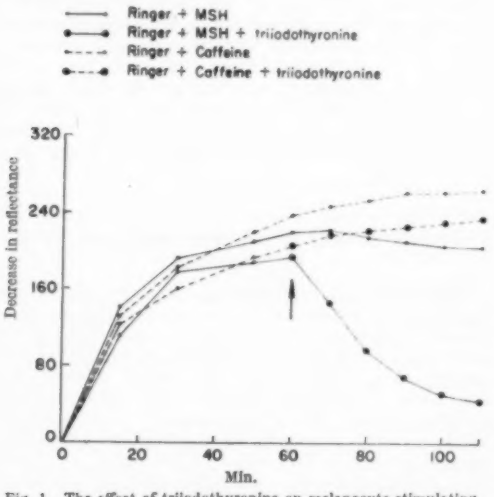

Fig. 1. The effect of triiodothyronine on melanocyte-stimulating hormone- and caffeine-darkened frog skin. Four comparable groups of skins were darkened, two with melanocyte-stimulating hormone and two with caffeine. Decrease in reflectance was followed. After 60 min. triiodothyronine (5 μgm./ml.) was added to one melanocyte-stimulating hormone- and to one caffeine-treated group. Triiodothyronine reversed the darkening action of melanocyte-stimulating hormone but not that of caffeine

The minimum amount required to initiate lightening was 0·5 μgm. per ml. of sodium triiodothyronine, 1·5 μgm. per ml. of thyroxine sodium pentahydrate and 25 μgm. per ml. of the diethanolamine salt of triiodothyroacetic acid. These potency ratios are in approximate agreement with those representing the thyroid hormone actions of these compounds as measured by inhibition of iodine uptake[2].

Melatonin, noradrenaline, serotonin and hydrocortisone can reverse the darkening actions of both melanocyte-stimulating hormone and caffeine. The only compounds known to reverse the melanocyte-stimulating hormone effect but not that of caffeine are acetylcholine and thyroxine and its two analogues[3].

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Immunochemical Observations on Antisera to Purified Human Growth Hormone

Recently, Hayashida and Li[1] and Read[2] have reported that purified preparations of human growth hormone were antigenic when injected with adjuvants into rabbits. Serum antibodies were demonstrated by the precipitin ring test[1] and by a modification[2] of the tanned cell hæmagglutination method of Boyden[3]. It was also shown that the rabbit antiserum inhibited the biological activity of human growth hormone[1]. Read[2] has employed rabbit antiserum to measure the concentration of growth hormone in human serum by the hæmagglutination–inhibition reaction[3,4]. It seemed essential in assessing the specificity of this method to investigate the purity of the antigen and the specificity of the antisera. In this report immunochemical evidence is presented which confirms that human growth hormone is a potent antigen. In addition, results were obtained which indicate that the Raben preparation of human growth hormone[5] is a highly purified antigen.

A relatively potent antiserum was prepared by the injection of rabbits with human growth hormone prepared by Raben (*Homo* No. 6) according to the following schedule[1]: 2 mgm. of human growth hormone in complete Freund's adjuvant (Difco) was injected both subcutaneously and intraperitoneally on the first and third weeks; on the fifth week human growth hormone was suspended in 1 per cent potassium alum[6] and 1 mgm. was injected by both the subcutaneous and intraperitoneal routes. The total amount of antigen was 10 mgm. per rabbit. The individual rabbit antisera were preserved by the addition of 1 per cent merthiolate to a final concentration of 1 : 20,000 after passage through a 'Millipore' filter.

The immunological specificity of the reactants was studied (1) by passive hæmagglutination and the hæmagglutination–inhibition reaction using tanned sheep erythrocytes conjugated with human growth hormone[2]; (2) by the double diffusion gel plate

technique of Ouchterlony as modified by Halbert *et al.*[7]; (3) by the Preer micromethod[8] for double diffusion in agar; (4) by Scheidegger's micromodification[9] of the immuno-electrophoresis procedure of Grabar and Williams; and (5) by the quantitative precipitin method, the nitrogen content of the precipitates being determined by the Markham micro-Kjeldahl method[6]. Human growth hormone from the same lot (*Homo* No. 6) was employed in experiments in Read's and in our laboratories.

The passive hæmagglutination titres of individual rabbit antisera ranged from 1/6,400 to 1/51,200. As little as 1·35 mμgm. nitrogen of antigen was detectable by Read's modification of the hæmagglutination-inhibition reaction. A single band of precipitation was obtained in Ouchterlony plates when undiluted antiserum was tested against dilutions of human growth hormone of 1 mgm./ml. to 10^{-2} mgm./ml. The intense band was completely removed following absorption of antiserum with human growth hormone. Complete fusion of single bands was demonstrated when antiserum was set up against 500 μgm./ml. dilutions of Raben's human growth hormone and Li's human growth hormone[10], a crude preparation of human pituitary, and simian growth hormone. No spur formation to indicate partial identity was found. A band of precipitation was not detected in the agar plates against 2 mgm./ml. of human adrenocorticotrophic hormone (Organon), bovine growth hormone, human chorionic and menopausal gonadotropins, or samples of unabsorbed human serum. Similarly, in studies of the antisera and of purified human growth hormone and crude pituitary extract, and of simian growth hormone by the Preer method, a single disk-like band of precipitation was obtained; the equivalence point was noted at a half dilution of antiserum against 62·5 μgm./ml. of human growth hormone (Fig. 1).

Investigation of the reactants by the immuno-electrophoresis procedure showed, in experiments in which the anti-human growth hormone serum was added to a longitudinal central trough and 500 μgm./ml. of human growth hormone to one lateral well and 1 mgm./ml. of a crude pituitary extract to the other lateral well, a single arc of precipitation against each preparation (Fig. 2, bottom). In this system human growth hormone exhibited the relative mobility of a slow β-globulin. The antigen did not react with horse hyperimmune anti-human serum (Fig. 2, top) or rabbit anti-human globulin. These studies indicate that contamination of purified human growth hormone with serum proteins is negligible. Since antibodies to human growth hormone were not detected in the Paris anti-human serum, it is quite reasonable to assume that the amount of human growth hormone contained in normal human serum is insufficient to elicit antibodies in the horse. The lack of cross-reaction between human growth hormone antiserum and human adrenocorticotrophic hormone (2 mgm./ml.), bovine growth hormone (1 mgm./ml.), and several human sera was confirmed by immuno-electrophoresis. Interestingly, the single, smooth, narrow arc of precipitation found with simian growth hormone revealed a slightly different electrophoretic mobility than did that obtained with human growth hormone, being situated slightly closer to the cathode.

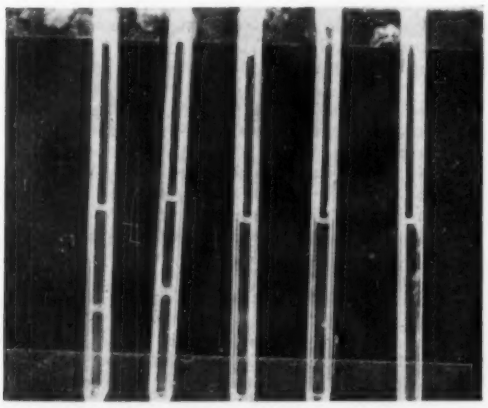

Fig. 1. Preer tubes with rabbit anti-human growth hormone serum in dilutions (from left to right) of undiluted, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ and $\frac{1}{16}$ in which the concentration of growth hormone added to the tubes was kept constant at 62·5 μgm./ml. Equivalence point at $\frac{1}{2}$ dilution of antiserum with single band of precipitation

Fig. 2. Top, immuno-electrophoretic precipitin pattern of human sera (upper well) with horse hyperimmune anti-human serum (Paris serum No. 491). No reaction noted between human growth hormone (lower well) and 'Paris serum' (longitudinal trough). Bottom, immuno-electrophoretic precipitin pattern of human growth hormone (500 μgm./ml.) (lower well) with rabbit antiserum (longitudinal trough). No reaction of antiserum with human adrenocorticotrophic hormone (2 mgm./ml.) (upper well)

The quantitative precipitin method was used to study the total amount of antigen–antibody precipitated by the antiserum after incubation with varying concentrations of antigen. Preliminary results indicate a precipitin curve consistent with a single antigen–antibody system.

It was also shown that the injection of the same antisera used in the studies described above and human growth hormone by separate routes into hypophysectomized rats completely inhibited the biological activity of the hormone, as assayed by weight gain and by the tibia test.

The results of these experiments indicate that the Raben preparation of human growth hormone is a potent antigen with a high degree of immunological homogeneity. Antisera prepared by injection of human growth hormone into rabbits could not be shown to contain precipitins or other antibodies specifically related to human antigens other than human growth hormone. Evidence was obtained of only a single antigen–antibody system. It seems almost certain from the results that the single system observed actually represents the human growth hormone–anti-human growth hormone reaction. However, the limitations of the immunochemical methods do not exclude the possibility that this preparation of

human growth hormone (*Homo* No. 6) is contaminated with trace amounts of other antigens or with weak antigens or, of course, with non-antigenic material.

The physicochemical and structural characteristics of simian and human growth hormone show important similarities[11] and both hormones exhibit biological activity in man. The different electrophoretic mobility of these two antigens was clearly shown by immuno-electrophoresis.

The apparent immunological specificity of purified human growth hormone and its antibodies strongly supports the usefulness of the hæmagglutination-inhibition reaction as a specific method for the quantitative estimation of growth hormone. Evaluation of the Read method as applied to human sera is in progress in this laboratory.

Note added in proof. In additional experiments in which human adrenocorticotrophin prepared by M. S. Raben was used, this material was found to be contaminated with small amounts of human growth hormone, in contrast to the highly purified Organon preparation employed in previous studies.

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Effect of Heavy Water on Different Types of Muscle and on Glycerol-extracted Psoas Fibres

On considering the suggestion that more attention be directed to the role of water in biochemical reactions[1], the possibility was entertained that by using heavy water further information might be obtained about physical-chemical processes in muscle. An investigation was therefore undertaken to observe the effects of heavy water on heart, intestinal, uterine and voluntary muscle.

Concentrations of heavy water between 99·8 and 25 per cent decreased the force of contraction and the rate of the spontaneously beating, isolated atria of the frog (*Xenopus laevis*). Similar effects have been observed previously on the frog heart[2]. The onset of the effects on the atria of *Xenopus* was noted 50 sec. after immersion in heavy water-Ringer solution. With 99·8 per cent heavy water, the force decreased by 90 per cent of its basal value after 2½ min. In

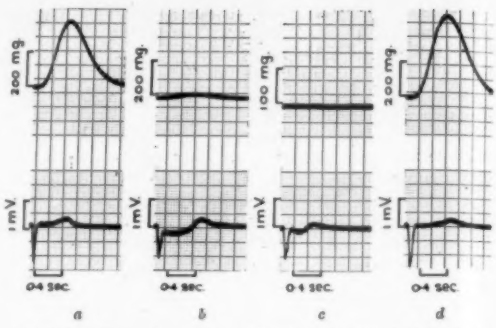

Fig. 1. Simultaneous recordings of tension (upper tracing) obtained with an RCA transducer and unipolar surface electroatriogram (lower tracing). *a*, Basal recording in normal water–Ringer; *b*, after 5 min. in 99·8 per cent heavy water–Ringer; *c*, after 17 min. in 99·8 per cent heavy water–Ringer (note no recordable contraction with increase in gain of amplifier); *d*, recovery after 15 min. in normal water–Ringer (note that the force of contraction is greater than in the basal recording)

most instances the atria either stopped beating or slowed down to one impulse every 5 or 7 min. after being immersed for about 20 min. in heavy water. The velocity of contraction was reduced[3], as was also the velocity of relaxation (Fig. 1).

Simultaneous recordings of the surface electroatriogram (Fig. 1) revealed that 99·8 per cent heavy water reduced the amplitude of the depolarization wave by about 13 per cent on the average. On the other hand, the amplitude of the repolarization wave was increased after 5 min. and then decreased towards the basal value; changes in the configuration of the repolarization wave were obvious. The duration of depolarization and repolarization increased by approximately 40 and 60 per cent, respectively. The interval between the onset of the electrical impulse and the contraction increased by about 90 per cent of the basal value. These manifestations in the mechanical and electrical parameters were reversed after transferring the atria to normal water-Ringer solution.

A particularly interesting feature was the dissociation in response of the electrical and contractile events. In 4 out of 10 experiments the contraction was not detectable while the impulse continued to be propagated (Fig. 1). Hodgkin and Horowicz produced similar dissociation of the twitch and action potential of single muscle fibres with high concentrations of sodium chloride[4].

After these preliminary experiments, attempts were made to elucidate the sites where heavy water affected the contraction of muscle. First, electrical stimulation of the heavy water-inhibited atria resulted in an action potential. The contractile response was, however, small and never greater in force than that seen prior to the arrest of the heart. It appears, therefore, that the membrane of the muscle fibres of the atria remained excitable while the spontaneous impulse was suppressed by inhibition of the pacemaker by heavy water.

Secondly, chemical stimulants such as adrenalin, noradrenalin and veratrine increased the force of contraction of atria treated with 75 per cent heavy water. In passing, it may be mentioned that heavy water also diminished the force of contraction of isolated rabbit duodenum. However, 75 per cent heavy water did not modify the effects of acetylcholine on rabbit duodenum and of oxytocin on the guinea pig's uterus. A high concentration of calcium

chloride (4·5 m*M*), sufficient approximately to double the force of contraction in untreated atria, had no inotropic effect on atria treated with heavy water. Whereas in untreated atria both adrenalin and noradrenalin increased the rate of rhythmic contraction, this chronotropic effect did not occur in the presence of heavy water.

After establishing that heavy water decreased markedly the force of contraction of voluntary muscle (rectus abdominis of frog and rabbit psoas), a third series of investigations was carried out with heavy water on psoas muscle fibres extracted with glycerol[5]. In the case of these glycerol-extracted fibres, however, heavy water did not retard the degree of shortening induced by adenosine triphosphate. To increase the probability of deuterium exchange, living fibres were activated by electrical stimulation while submerged in heavy water and then extracted in 99·8 per cent heavy water at 4° C. instead of in 50 per cent glycerol. The response of the fibres treated in this way was again not affected by adenosine triphosphate dissolved in heavy water–salt solution. Further experiments on the glycerol-treated fibres with other nucleotides revealed that heavy water reduced the amount of shortening produced by inosine triphosphate and uridine triphosphate but not the amount produced by adenosine triphosphate and cytodine triphosphate (Fig. 2). In so far that the difference in molecular structure between inosine triphosphate and adenosine triphosphate on one hand and between uridine triphosphate and cytodine triphosphate on the other is in the 6th position of the purine and the pyrimidine rings respectively, it is suggested that reactions involving the 6th position of either inosine triphosphate or uridine triphosphate were retarded by deuterium.

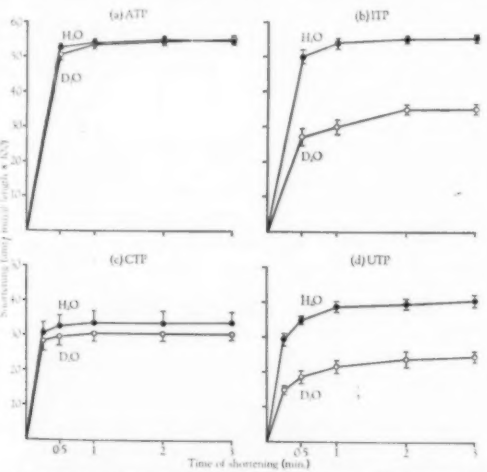

Fig. 2. The effect of heavy water on the degree of shortening of glycerol-extracted psoas fibres in the presence of a nucleotide. Each fibre was soaked either in normal water – salt or heavy water – salt (*p*H about 6·8) for 30 min. prior to immersion in the particular nucleotide solution. The salt solution contained magnesium chloride (0·001 *M*) and potassium chloride (0·01 *M*) except in the case of (*b*) where the magnesium chloride concentration was 0·01 *M*. Average values (with standard errors) are depicted of ten experiments with normal water – salt – nucleotide solutions (●) and heavy water – salt – nucleotide solutions (○). *a*, 3·3 m*M* adenosine triphosphate (*p*H 3·6). (In experiments with 1·7 m*M* concentration of adenosine triphosphate or at neutral *p*H, heavy water likewise had no retarding influence); *b*, 3·3 m*M* inosine triphosphate (*p*H adjusted to 7·1). (Heavy water similarly had a retarding influence with inosine triphosphate at higher concentrations or at a lower *p*H and lower magnesium chloride concentration); *c*, 6·6 m*M* cytodine triphosphate (*p*H 4·35); *d*, 6·6 m*M* uridine triphosphate (*p*H 3·56). (Insufficient material precluded studies on uridine triphosphate and cytodine triphosphate at varying concentrations and *p*H's)

As the degree of shortening of the contractile proteins was not necessarily reduced by heavy water, the possibility exists that either the substitution of deuterium for hydrogen in the contractile proteins did not in itself retard the response or that little exchange took place. Taken in conjunction with the observed dissociation of the electrical and contractile events in the living muscle, it seems likely that the energy transfer mechanism between the membrane and the contractile protein is retarded or blocked by the isotopic effect of deuterium. The response produced by the drugs, for example adrenalin, could be due to the activation of the energy transfer process. Calcium ions, on the other hand, could not counteract the effects of heavy water. The manner whereby heavy water inhibited the pacemaker remains to be elucidated.

It thus appears that heavy water can be useful as a tool in the dissection of the physical-chemical processes of muscle contraction. Full details of the experiments will be published elsewhere.

Grateful acknowledgement is made to Prof. J. Gillman for his helpful encouragement and critical interest. My thanks are due to Dr. S. Brenner for suggesting the use of heavy water, and for helpful discussion, to Drs. P. R. Levy and A. G. Szent-Györgyi for advice, and to Mr. A. J. Oliphant for invaluable technical assistance.

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Effect of Parathyroid Extract on Citric Acid Concentration in the Rat Kidney

THE influence of parathormone on the level of citric acid in plasma was first mentioned by Sjöström[1], who found an elevated level of citric acid in patients with hyperparathyroidism; this finding was afterwards confirmed by other authors. The effect of parathormone on the citric acid level was verified by Neuman *et al.*[2], who measured the amount of the acid in the venous blood of bones and found an elevated level after the application of parathormone. The purpose of the work reported in this communication was to prove this effect of parathormone on the metabolism of citric acid also in other tissues and to try to explain the mode of action.

The concentration of citric acid in the skeleton and kidneys of female rats, 150–160 gm., was observed. The rats received 10 u. of parathyroid extract ('Parathyroid', Paines and Byrne) intramuscularly, and were killed at intervals of 1–24 hr. after the injection. The kidneys were immediately homogenized in liquid air; the dissected left femur was extracted for 7 days in a solution of 10 per cent trichloracetic acid. Citric acid was determined by Gey's method[3]. In order to explain the mode of action of parathormone, the parathyroid extract was given

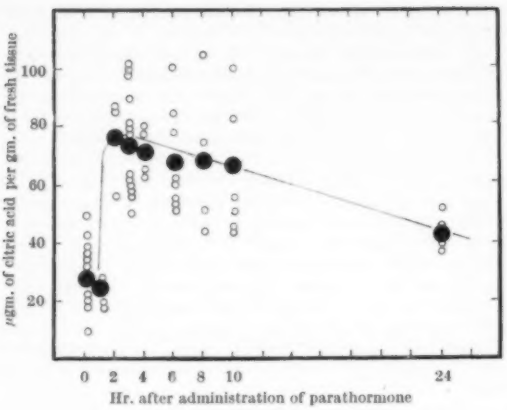

Fig. 1

3 hr., and a freshly prepared sodium fluoracetate solution (5 mgm./kgm. body-weight) was given 1 hr., before the animal was killed, to obtain the maximal effect of both compounds.

The results are presented in Table 1 and Fig. 1. The extract of parathyroid glands raises sharply the concentration of citric acid in the kidneys after latent periods of 60 min. The elevation of citric acid is statistically significant ($P \ll 0{\cdot}01$) in comparison with the control group. It reaches its maximum in 2–3 hr. after the injection. The concentration of citric acid remains at approximately the same level up to 10 hr. after the injection and returns to about the starting concentration after 24 hr.

Table 1. Concentrations of Citric Acid mgm./gm. of Fresh Tissue in Different Time Intervals after the Application of Parathyroid Extract

Control	1 hr.	2 hr.	3 hr.	4 hr.	6 hr.	8 hr.	10 hr.	24 hr.
0·019	0·030	0·076	0·10	0·065	0·060	0·044	0·096	0·044
0·023	0·028	0·085	0·10	0·080	0·054	0·074	0·082	0·050
0·039	0·018	0·085	0·080	0·062	0·051	0·104	0·099	0·038
0·028	0·018	0·056	0·10	0·078	0·055	0·051	0·068	0·038
0·050			0·060		0·083		0·043	
0·043			0·080		0·10		0·056	
0·030			0·080		0·078		0·045	
0·010			0·050		0·061		0·050	
0·020			0·060					
0·033			0·090					
0·038			0·078					
0·021			0·056					
0·037			0·061					
0·035			0·063					
			0·059					
Mean values								
0·030	0·023	0·075	0·074	0·071	0·068	0·068	0·067	0·042

After the application of sodium fluoracetate, which inhibits the further degradation of citric acid in the Krebs cycle, the concentration of citric acid in the kidneys rises fifty times[4] within 1 hr. When sodium fluoracetate and parathormone were given so as to achieve the maximum effect of both compounds simultaneously, there was statistically no significant difference in the content of citric acid in the kidneys compared with the control group ($P = 0{\cdot}1$).

The content of citric acid in bones remained the same after administration of parathormone alone or in combination with fluoracetate; there was no significant difference between these groups.

Our findings point to the fact that parathormone influences the metabolism of citric acid not only in the bones but also in other tissues—in our case in the kidneys of rats. We assume that the parathormone may interfere directly with the Krebs cycle and that it may act either by raising the synthesis of citric acid or by inhibiting its degradation. The blocking of citric acid metabolism in the Krebs cycle by fluoracetate did not enhance the effect of the parathormone on the concentration of citric acid. It appears, therefore, that the interference of parathormone with the metabolism of citric acid is caused rather by inhibition of its further degradation than by augmentation of its synthesis. Similar effect has been described in the mode of action of vitamin D[5]. As, however, the quantity of citric acid is much higher after administration of fluoracetate than after parathormone, it should be taken into account that a raised synthesis of citric acid, owing to the effect of parathormone, can be hidden in the great excess of citric acid and that the relatively small changes induced by the parathormone are scarcely detectable by the method used. The same explanation can be applied to the negative response of bones to the administration of parathormone and fluoracetate because of high levels of citric acid in bones under normal conditions.

The question of the mode of interference of parathormone with the Krebs cycle is not fully explained by our findings and requires further investigation.

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Antithyroidal Effect of Some Fresh Plants on Guinea Pigs

Several investigators have described a goitrogenic effect of various *Brassica* plants (see Greer's review[1]). Papers have appeared in recent years dealing with the goitrogenic properties of milk from cows fed on various species of *Brassica*[2–5]. As, however, no goitrogenic substances had until recently been proved in the edible parts of *Brassica* (except in rutabaga (*Brass. napus* var. *esculenta*) and turnip[1]) quite a number of problems remained unanswered. Recently, Altamura *et al.* isolated L-5-vinyl-2-thio-oxazolidone also from fresh cabbage[6]. On the basis of our previous studies[5] we decided to study the effect of *Brassica* and other plants on the thyroid gland of laboratory animals with the aid of newer methods.

12 male guinea pigs were divided into three groups (4 animals per group) and maintained 48 hr. on tap-water only. Then they received vegetables (group I carrot, group II kohlrabi and group III kale). After 24 hr., in which they consumed approximately 70–

80 gm. of vegetables per animal, they were injected with 3 μc. iodine-131 in 0·5 ml. of physiological saline intraperitoneally. 4 hr. later they were killed under ether anæsthesia. Radioactivity of thyroid glands was determined after hydrolysis in 30 per cent sodium hydroxide with the aid of a scintillation counter and the thiocyanate-level in serum was determined by Aldridge's method[7].

Table 1. Effect of Certain Plants on Accumulation of Iodine-131 on the Thyroid Glands and on Thiocyanate-level in Serum

Plant	Iodine-131 in thyroid gland (c.p.m./mgm.)	Thiocyanate in serum (mgm. per cent)
I Carrot	5,549 ± 994	0·053 ± 0·012
II Kohlrabi	3,343 ± 636	0·550 ± 0·161
III Kale	2,778 ± 601	1·605 ± 0·807

Accumulation of radioiodine after feeding kohlrabi and kale was significantly lower than after feeding carrot ($P < 0·02$). Kohlrabi and kale were used because of their different contents of thiocyanate[8]. The results indicated that thiocyanate may indeed be a cause of the antithyroidal effect of these plants, but some other agent must likewise be present. Further experiments are in progress.

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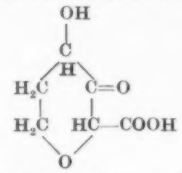
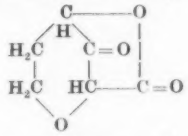
Activity of a Substance extracted from the Fermentation Products of *Acetobacter aceti* on Ehrlich's Ascites Tumour Cells

During the bacterial fermentation of alcohol to acetic acid, highly active molecules capable of regulating oxidation-reduction potentials must be elaborated by the bacteria, but as yet no extensive investigations in this field have been carried out. A substance possessing interesting biological activities and with some well-defined chemical characteristics has recently been isolated from vinegar[1,2]. This substance, obtained by extraction and purification processes similar to those used for cytochrome isolation, proved to have a marked oxidizing ability and, in particular, exerted an intense facilitatory activity on some catalase systems. It also markedly inhibited the growth of human tumour cells cultivated *in vitro*. When the substance is added to the medium in very low concentration in *in vitro* respiration experiments (Warburg technique) with Ehrlich's ascites tumour cells, an increase of respiration occurs: on the other hand, a most important finding is that respiration is blocked at higher concentrations. This interference with respiratory processes is much less marked on non-tumour cells. These effects would support Antoniani's concept[3] of the extreme sensitivity of tumour cells to substances capable of interfering with their respiratory systems, which, owing to discontinuous pathways, are particularly labile.

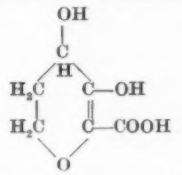
The substance, previously characterized by extraction methods and chromatographic purification, has been recently isolated in the pure state. It is a white powder with a melting point of 184° C. Its probable structural formula, derived from the elementary analytical data, from determination of the molecular weight (camphor method), infra-red spectrum, chemical groups and break-down studies, is:



It may be in equilibrium with a γ-lactone form:



or may exhibit an enolic form as follows:



The compound is easily soluble in water; the solutions are acid (about *p*H 3·2) and slightly yellow.

Its toxicity is very low: 2·5 gm./kgm. intravenously are not lethal for mice. The addition of the compound (concentration 1 : 100,000) to the perfusing fluid in the Straub frog heart preparation induces a digitalis-like pattern without producing heart block.

Two types of experiment have been performed with samples of the pure substance in saline adjusted to *p*H 7·4.

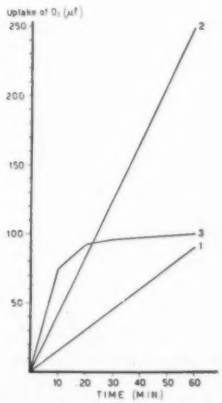


Fig. 1. 1, Ehrlich ascites cells, 1·0 ml. (2·9 mgm. nitrogen by Kjeldahl); ascites serum, 1·1 ml.; glucose solution, *M*/100, 0·5 ml.; substance, 0
2, Ehrlich ascites cells, 1·0 ml. (2·9 mgm. nitrogen by Kjeldahl); ascites serum, 1·1 ml.; glucose solution, *M*/100, 0·5 ml.; substance, 0·6 mgm.
3, Ehrlich ascites cells, 1·0 ml. (2·9 mgm. nitrogen by Kjeldahl); ascites serum, 1·1 ml.; glucose solution *M*/100, 0·5 ml.; substance, 3 mgm.

Table 1. Mice with Ehrlich's Ascites Tumour

No. of animals		Days after tumour transplantation					
		0	2	4	6	8	10
30 controls	body-weight	19·60	20·10	23·10	25·60	26·9	31·2
	cells/ml. present in ascitic fluid	25,000,000	89,600,000	146,700,000	165,800,000	—	—
30 treated (3 mgm./day of the substance)	body-weight	19·83	20·02	20·20	21·70	21·3	22·3
	cells/ml. present in ascitic fluid	25,000,000	38,450,000	27,650,000	37,900,000	—	—

The effects *in vitro* on the respiration of Ehrlich's ascites tumour cells have been studied using the Warburg technique, while in the *in vivo* experiments Ehrlich's ascites tumour in the white mice were used.

The results of the *in vitro* experiments are illustrated in Fig. 1. When the compound is added, in doses of 0·6 mgm., to the fluid containing the ascites tumour cells (equivalent to 2·9 mgm. nitrogen) and substrates, made up to a total volume of 2·8 ml., a marked increase in cellular respiration is seen in the first hour. The addition of 3 mgm. of substance, under the same experimental conditions, produces increased respiration for the first 20 min. and then a complete inhibition of respiration.

The results of the *in vivo* experiments are reported in Table 1. The neutralized solution was injected daily at a dose of 3 mgm./animal/day beginning from the second day after the intraperitoneal inoculation of the ascitic fluid containing the tumour cells.

Treated mice showed nearly complete inhibition of tumour growth (there was no increase in body-weight) and very marked decrease of number of cells per ml. of ascitic fluid.

These results confirm the marked *in vivo* activity of this new substance in inhibiting the development of the particular type of tumour cell used in these experiments.

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BIOPHYSICS

Reversible Supercontraction of β-Keratose

Peacock[1] has examined the X-ray diffraction patterns from Lincoln wool supercontracted by oxidation in peracetic acid. An observation made by Whiteley[2] using Alexander's[3] peracetic acid–ammonia technique to separate the protein components in wool[4] was that, following supercontraction in peracetic acid, the fibres increase in length when the α- and γ-keratoses are dissolved out by the ammonia solution. Mercer found it possible to prepare a birefringent fibre from the protein extracted from wool by urea-reducer solutions which showed dimensional changes as the *p*H was altered[5].

We now find that β-keratose, which remains as a continuous fibre containing some 10 per cent of the original protein[3], will contract again if taken from alkaline solution into solutions of lower *p*H. Moreover, the change in length of the β-keratose fibre as the *p*H is altered is reversible, without hysteresis, and can be repeated many times with a single fibre. The experiment has been carried out with oxidized Merino and Lincoln wools, human hair and horse hair at temperatures between 30° and 60° C.

In a typical experiment a single horse hair about 2 cm. long was mounted in a setting frame and oxidized for 17 hr. at 40° C. and 12 hr. at 60° C. by an approximately 5 per cent w/v peracetic acid solution. The hair was then extracted with 0·1 *N* ammonia and contraction-extension experiments were carried out by placing the extracted fibre in solutions of various *p*H values made from mixtures of 0·01 ammonia and 0·01 *N* acetic acid. The length of the fibre was measured with a travelling microscope after equilibration at each new *p*H at 23° C. for ½ hr. The results are shown in Fig. 1.

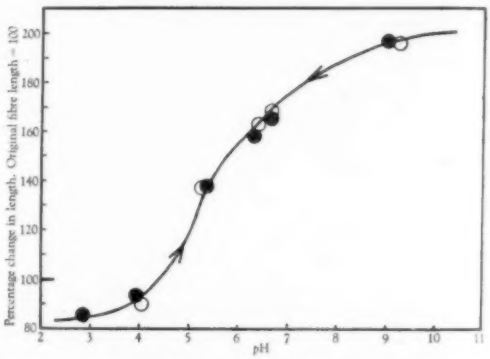

Fig. 1. Reversible length change of β-keratose from horse-hair as the *p*H is altered. Full circles: *p*H falling; open circles: *p*H rising

When examined by X-ray diffraction, Lincoln wool appears to be exceptional in giving a pattern indicative of α-protein both after mild oxidation (see Peacock[1], for example) and after contraction of the alkali-extended fibres. On the other hand, prolonged oxidation results in the appearance of a pronounced β ring at 4·65 A., and this change in the diffraction pattern, often accompanied by some preferential orientation on the meridian (the cross-β pattern) associated with the supercontracted state, is the more usual observation in other keratin fibres oxidized with peracetic acid. After extraction of these oxidized fibres with ammonia, the insoluble residue, when alkaline, always gives only a diffuse protein-type pattern in spite of attempts to orient the material by stretching. As the *p*H is lowered, a pronounced sharpening of the 4·65 A. reflexion develops and this shows a slight preferred orientation on the meridian after carefully stretching the wetted fibres. These

changes have been followed particularly in the more robust horse hair. In contrast to these findings, neither dimensional nor configurational changes have been observed in thin films of α-keratose cast from ammonia (they give a well-oriented α-pattern) and subjected to the same changes of *p*H as those applied to the extracted fibre.

The relationship between supercontraction in wool and hair and the contraction of muscle has been repeatedly pointed out by Astbury[6,7], and there is evidence that supercontraction occurs during the syneresis of actomyosin gels[8]. Moreover, the ease with which actomyosin undergoes supercontraction in the presence of hydrogen ions[9] supports the evidence of Goodall[10] that a proton-transfer mechanism might be responsible for the development of tension in muscle. The dimensional changes of β-keratose as the *p*H is altered suggest again at least a partial contraction to the cross-β configuration, though apparently in this case, since the X-ray diagram given by the extended fibre is so diffuse, not from a well-defined α-configuration but from some intermediate less-regular state.

We wish to thank Prof. W. T. Astbury for his advice and encouragement in this investigation, which is supported in part by the Muscular Dystrophy Associations of America, Inc., and will be reported in detail elsewhere.

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Leeds, 2.
Oct. 6.

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PLANT PHYSIOLOGY

Effect of Infection by *Xanthomonas malvacearum* (E.F.S.) Dowson on Respiration-rate of Cotton Leaves

Potted plants of susceptible Domains Sakel cotton (*Gossypium barbadense*, L.) and its synthesized blackarm-resistant strains containing B_2 or $B_2 + B_3 + B_6$ genes were sprayed when about three months old with a standardized water-suspension of *Xanthomonas malvacearum* taken from three-day-old cultures of a recent isolate. The plants were kept in the shade under glass bell-jars for 48 hr. after inoculation. The leaves used for determining respiration-rates were chosen from among fully expanded leaves 5 cm. in diameter situated at the ninth to thirteenth nodes of the stem.

The number of spots on inoculated leaves ranged from 80 and 200 per leaf. A Thoday respirometer was used for determining oxygen uptake, which was taken as a measure of respiration-rate. Two leaves, of about 1 gm. fresh weight, were placed in each respiratory chamber with the petioles dipped in distilled water in a specimen tube. The respiratory chamber also contained 10 ml. of 5 per cent potassium hydroxide and a piece of Whatman folded filter paper No. 40 to increase the absorption surface. The chambers were placed in a temperature-controlled water-bath adjusted at 30° C. Readings were taken 2 hr. after the experiment had been started and continued thereafter at 8-hr. intervals. The first reading in all experiments was discarded to avoid misinterpretation of handling effects.

In 1957, two sets of experiments were carried out using leaves of susceptible Domains Sakel. In the first set, uninoculated (control) leaves as well as leaves 4 and 10 days after inoculation were used. The second set included control leaves, and leaves taken 10 and 16 days after inoculation. The experiments were run for 2 days. The respiration-rate of the leaves of resistant strains of Domains Sakel containing B_2 and $B_2 + B_3 + B_6$ was also examined in uninoculated leaves (control) and in leaves 10 and 16 days after inoculation. These experiments were run for 4 days.

In 1958, experiments were carried out with the susceptible Domains Sakel using uninoculated leaves (control) and leaves 4, 8, 12 and 16 days after inoculation. The leaves were cut at noon, washed thoroughly with distilled water, blotted dry with filter paper, weighed and placed in the respiratory chamber. The experiments were run for 4 days and thus respiration was actually measured continuously from the fifth to the twentieth day after inoculation.

Angular leaf spots first appeared on susceptible Domains Sakel 5–7 days after inoculation as minute, water-soaked, dark green spots mainly on the under-surface of the leaf. The spots increased in size, becoming 2–4 mm. in diameter in a few days, but did not attain their characteristic brown colour until 15–16 days after inoculation. On the resistant strains of Sakel, development of disease symptoms could only be ascertained between the eighth and tenth day after inoculation, when minute, pin-head, dark green, dry round spots appeared. The spots turned dark brown with age but remained very small.

The results of experiments in 1957 (Table 1) showed that there was a general depression in the respiration-rate of the inoculated leaves of susceptible Domains Sakel and its resistant strains compared with the controls. The depression seemed to reach its maximum in susceptible Sakel earlier than in the resistant strains.

The picture was clearer in the 1958 experiments, where the respiration-rate of Domains Sakel leaves was followed up from the fifth to the twentieth day after inoculation. Fig. 1 shows that maximum depression in respiration-rate was reached between the fifth and seventh days after inoculation. Respiration-rate then increased continually until the twentieth day, when no further readings were recorded.

Fluctuations in respiration-rate seem to coincide with the development of disease symptoms. Thus the early depression in respiration coincides with the period in which the bacteria enter the stomata and multiply in the stomatal chambers, blocking the stomata and suppressing gas exchange. This phase of the course of disease takes place in both susceptible and resistant strains of Domains Sakel. About the time disease symptoms first appear as pinhead spots, respiration reaches its minimum (that is, 5–7 days

Table 1. Respiration-Rate of Leaves of Resistant and Susceptible Strains of Domains Sakel Cotton inoculated with *X. malvacearum* (1957 Experiments)

Cottons	Oxygen uptake (ml. dry gas/10 gm. fresh wt./hr.)			
	Days after inoculation			
	Control	4	10	16
Susceptible Domains Sakel				
Means of three experiments	6·15	5·28	5·90	—
Difference from control (per cent)		−14·2	−4·1	—
Means of three experiments	5·71	—	5·36	7·88
Difference from control (per cent)			−5·1	+38·00
Sakel containing B_2				
Means of two experiments	5·39	—	4·79	4·88
Difference from control (per cent)		—	−11·1	−9·5
Sakel containing $B_2+B_3+B_4$				
Means of two experiments	5·39	—	4·62	5·53
Difference from control (per cent)		—	−14·3	+2·6

after inoculation in the case of susceptible Sakel and later in that of resistant strains). The increase in respiration-rate of the leaves of susceptible Domains Sakel that follows coincides with the increase in the size of the spots and the disintegration and collapse of the spot cells. The increase may be attributed partly to the evolution of the gas previously accumulated in the tissues, and partly to increased respiratory activity of the cells resulting from infection. The amount of gas contributed by the bacteria themselves to carbon dioxide output of affected leaves is difficult to assess but is unlikely to be of much significance.

In the resistant strains, the spots remain pinhead in size and the respiration-rate increases to about normal after their appearance. In this instance the depression in carbon dioxide output is less than in susceptible Sakel, but continues for a longer period before it returns to more or less the normal rate.

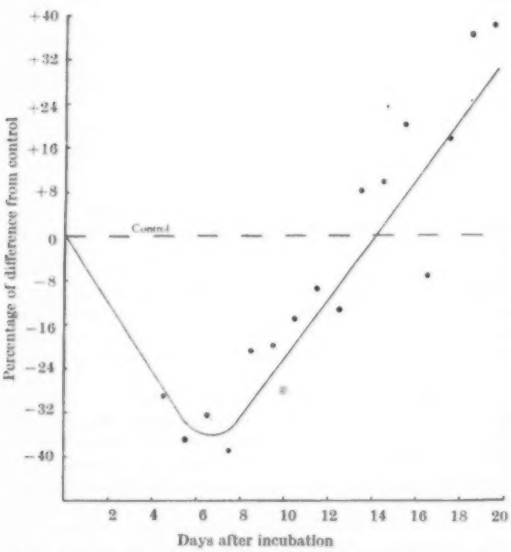

Fig. 1 Relationship between respiration-rate and age of inoculation

The damage caused to the host is little, if any, and does not provoke marked cell respiration.

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Chemical Nature of the Cell Wall of the Green Algae, *Codium*, *Acetabularia* and *Halicoryne*

The chemical nature of the carbohydrates which constitute the main cell wall of the algae belonging to Codiaceae and Dasycladaceae has remained thus far largely obscure. Though the cell wall of *Codium* was said to consist of callose and pectin together with some cellulose[1], no chemical characterization of callose and algal pectin has yet been achieved, their occurrence being recognized merely by staining reactions and solubility tests. Likewise, no unequivocal proof for the presence in *Codium* of true cellulose has hitherto been obtained, although its occurrence has occasionally been reported[2,3]. As for the cell-wall constituents of the members of Dasycladaceae almost nothing is known at present except the incrustation of calcium in the cell wall.

As a continuation of the biochemical researches of algal cell wall by one of us[4], we have been engaged in the study of the cell-wall constituents of the green algae from different taxonomic groups with the view of obtaining information about the phylogeny and systematic position.

The present communication deals with the studies on *Codium fragile*, *Acetabularia calyculus* and *Halicoryne wrightii*.

The air-dried sample of each seaweed was soaked in 1 per cent hydrochloric acid for 24 hr. at room temperature to remove calcium carbonate. It was then treated successively with 1·25 per cent sodium hydroxide and 1·25 per cent sulphuric acid at 100° for 30 min. and the residue was bleached with 1 per cent sodium chlorite. The crude fibre thus obtained retained the membrane structure almost unchanged and showed neither anisotropy nor staining reactions for cellulose.

Hydrolysis of the crude fibre with sulphuric acid and examination of the products by paper chromatography revealed the main constituents to be mannose. A trace of glucose as detected in the hydrolysate arose presumably from starch which could not be removed by the above treatment. The crude fibre is insoluble in sodium hydroxide at any concentrations, but easily soluble in 50 per cent zinc chloride, less in 50 per cent stannic chloride and slightly in Schweizer's reagent. Hence the purification was effected by dissolution in zinc chloride followed by precipitation with acetone. As the product contained about 2 per cent of ash, of which the major part was silica, it was removed by the method of Feigl and Krumholz[5]. The purified substance is a white powder with an ash content less than 0·1 per cent. Elementary composition corresponded to hexosan. Calculated for $C_6H_{10}O_5$, C 44·44, H 6·22; found, C 44·23, H 6·09 (*Codium*); C 44·24, H 6·17 (*Acetabularia*); C 44·31, H 6·12 (*Halicoryne*). $[\alpha]_D$ in 50 per cent zinc chloride −46·1° (*c.* 1·41, *Codium*); −50·6° (*c.* 1·54, *Acetabularia*); −46·1° (*c.* 1·41, *Halicoryne*). Hydrolysis with 40 per cent hydrochloric acid at 0° yielded 91–93 per cent of mannose. On the paper chromatogram no

other spots than that of mannose could be detected. Mannose phenylhydrazone (m.p. 199°) was obtained in nearly the theoretical yield[6]. When oxidized with periodate 0·96 mole of it was consumed and 0·125 mole of formic acid was produced per unit ($C_6H_{10}O_5$) of the preparation from *Codium*. Assuming a straight-chain structure with 1,4 linkage the yield of formic acid indicates a degree of polymerization of 16.

These results, taken together with the resemblance in specific rotation to the mannan of ivory nut (− 44·1°) and salep (− 35·0°), both of which are known to be β-1,4-linked, would indicate that the mannans from *Codium*, *Acetabularia* and *Halicoryne* are constructed in a similar manner.

Other *Codium* species such as *C. latum* and *C. tomentosum* also contain mannan as main cell-wall constituent, whereas in *Udotea*, *Chlorodesmis* and *Halimeda*, all of which are usually included in Codiaceae, mannan is replaced by xylan with β-1,3-linkage.

The significance of these findings in connexion with phylogeny and taxonomy will be discussed elsewhere.

We wish to express our gratitude to Profs. S. Inoh and S. Segawa as well as to Drs. M. Chihara and S. Kamura for their generous support in this work.

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BIOLOGY

Attrition of Incisors of Grazing Sheep

Under New Zealand conditions of sheep-farming, incisors of grazing sheep wear much more rapidly on improved pasture, chiefly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*), than on the finer native pastures of low carrying capacity. The cause is not nutritional in the generally accepted sense, however[1]. It would rather seem that certain substances in the herbage of 'improved' pastures dissolve the teeth, the process being aided by the abrasive action of the plant fibre. Wear is also increased in mouths with certain undesirable anatomical characteristics which appear to be hereditary in origin.

Differences in chemical compositions of enamels and dentines of sheep's teeth of various breeds and from farms on country of dissimilar soil types are not correlated with the extent of tooth wear[2].

The thin layer of enamel on the incisal surface of sheep's teeth is worn through approximately two months after eruption, hence the problem is concerned with the rate of wear of primary dentine and, at a later stage, secondary dentine.

Electron micrographs of the biting edge of worn incisors showed that the dentine surface was subjected to both chemical and abrasive action. The chemical action was thought to be due to the organic compounds in the herbage complexing the calcium phosphate (apatite), but before this could be checked by studies with the electron microscope it was essential to understand the microstructure of the organic and inorganic components of dentine more fully[3] and to develop a technique which enabled the intensity and nature of etching to be recorded (Hall, D. M., unpublished work). Experiments were then made to compare the etching produced by fractions of juices of pasture plants on surfaces of fresh dentine polished smooth by metallographic techniques. The fractions examined were: (1) organic acids from herbage at their original concentrations and *p*H (6·0–6·5) for chelating action on the calcium; (2) cell fluids, obtained by ether cytolysis, containing in addition sugars, inorganic and organic phosphates, amino-acids and polypeptides, capable of chelating or dissolving calcium; (3) freshly expressed juices of grasses and clovers containing enzymes in addition to substances of (1) and (2).

These studies have shown that fraction (3) produced the strongest etching, and indicated that this reaches a maximum during the lush spring growth. It was also evident that the central non-calcified core of the odontoblast process was etched by fresh grass juice at a faster rate than the dentinal matrix and thereby released long lengths of the membrane-like sheaths enclosing the process.

The results indicate that the majority of the etching is due to proteolysis and that such weakening of the bonding material of the tooth would facilitate its wear by attrition. Rouiller has observed that an organic component bonding the particles of dentine is digested by plant proteinases such as papain[4,5]. It is considered, therefore, that the digestive action of the proteinases of actively metabolizing leaves on the organic components of dentine could be an important factor contributing to the wear in teeth of grazing sheep.

This investigation is being continued.

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Rhizosphere Effect on Soil Algae

Very few studies of the effect of the rhizosphere on soil algae have been made in the past[1], and in a recent review Starkey[2] states that it is possible that algal populations may even be decreased in the root zone. This communication presents evidence for a well-marked rhizosphere effect on soil algae by the roots of cultivated tea plants nominally referable to the Assam variety of *Camellia sinensis* (L) O. Kuntze.

Roots from one-year old seedlings were removed from the nursery beds and the soil from the top 5 cm. of the roots was scraped off with a sterile spatula. The top 5 cm. of soil from adjacent bare areas of the same bed was used as the control. Modifying the method described by Agnihothrudu[3], 5 gm. each of

Table 1. Presence or Absence of Algae in Various Dilutions of Bristol's Solution

	Dilution						
	1/10	1/100	1/1,000	1/10,000	1/100,000	1/1,000,000	1/10,000,000
Rhizosphere soil	+	+	+	+	+	+	+
Control soil	+	+	+	+	+	−	−

control and rhizosphere soil samples were used for preparing a series of ten dilutions in Bristol's solution[4], the initial dilution being 1/10, and each higher dilution made by taking 10 ml. of the lower dilution and adding it to 90 ml. of sterile Bristol's solution. Three replications of each dilution were made and growth was estimated after three months by the presence or absence of algae in the flasks (Table 1). Previous observations on the soil algae of our sub-tropical soils showed that this period was sufficient for growth to occur and also that Bristol's solution was the most suitable of several media tried.

There is at present no reliable method of estimating numbers of soil algae in a mixture of unicellular, colonial and filamentous forms and the presence or absence of visible growth remains the only satisfactory method for this type of study.

These results give evidence of an effect of the rhizosphere on soil algae in the case of tea roots. The experiments have been repeated with other crop plants, both monocotyledons and dicotyledons, and have confirmed the presence of this effect. The results will be published in greater detail elsewhere.

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The Field-Slug *Agriolimax reticulatus* as a Vector of the Lungworm *Cystocaulus ocreatus*

Gerichter[1] demonstrated that the land snail *Monacha syriaca* can act as a vector for the sheep lungworm *Cystocaulus ocreatus*. Development of the lungworm larvæ can take place in other species of land molluscs and also in some freshwater species[1,2]. The importance of most of these molluscs as vectors under field conditions does not, however, appear to have been investigated.

C. ocreatus was recorded only recently in British sheep[3]. Afterwards a study of the transmission of infection under natural conditions was commenced at Weybridge. Lungworm-free lambs were grazed with infected ewes and, at frequent intervals, fæcal samples from the lambs were examined for first-stage lungworm larvæ. After several weeks, larvæ were recovered from the fæces, and on autopsy of one lamb, adult worms were found in the lungs. Samples of the land molluscs inhabiting the pasture grazed by the sheep were collected and examined for lungworm larvæ. The only species infected was the field-slug *Agriolimax reticulatus*, so that it appeared that infection of the lambs had resulted from the ingestion of field-slugs. To verify that this was so, the feet from artificially infected slugs were fed to two stalled lambs reared free of lungworms. After several weeks, first-stage larvæ of *C. ocreatus* were recovered from both lambs.

A. reticulatus was by far the most numerous of the mollusc species collected from the Weybridge pasture, specimens being found throughout the year. According to Ellis[4], the field-slug is the commonest and most ubiquitous of the land molluscs found in Britain, inhabiting every possible situation. As *A. reticulatus* is almost certain to be found wherever sheep can graze, it is most likely that it is of considerable importance as a vector of *C. ocreatus* in Britain.

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The Pituitary of *Myxine glutinosa*

The Myxinoids are possibly the most primitive living vertebrates, and the pituitary of *Myxine glutinosa* has hitherto[1-3] been described as having a separate infundibulum and adenohypophysis, a condition which has been regarded as primitive or degenerate. Furthermore, the adenohypophysis which lies embedded as groups of cells in connective tissue, has been described as being composed solely of chromophobes, and having no cytological or histological differentiation. Recent observations, however, have shown that the connective tissue septum does not separate completely the two parts of the pituitary but that the posterior end of the infundibulum comes into intimate contact with the adenohypophysis (Fig. 1). Also, the adenohypophysis is cytologically differentiated.

An examination of the infundibulum using Bodian's stain indicated that nerve fibres lie between and beneath the ependymal cells and posteriorly are closely apposed to adenohypophysial cells. Using several methods to demonstrate neurosecretory material this was shown to be present in the infundibular canal particularly where it narrows posteriorly.

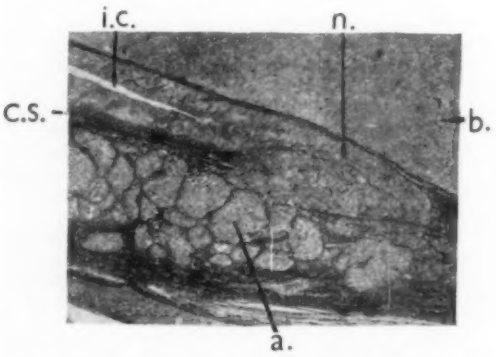

Fig. 1. Longitudinal section of the caudal end of the pituitary of *Myxine glutinosa* showing apposition of neurohypophysis and adenohypophysis. *a*, Adenohypophysis; *b*, brain; *c.s.*, connective tissue septum; *i.c.*, infundibular canal; *n*, neurohypophysis

Neurosecretion was also present in the region of the neurones lying outside the infundibulum. In the caudal part of what now must be regarded as a neurohypophysis a few cells identical with the ependymal cells are found separate from the lining of the ventricle. No neurosecretory material was found in the adenohypophysial region.

The adenohypophysis when stained with Heidenhain's azan stain or Mallory's triple stain was shown to contain at least two different types of basophils in addition to chromophobes and large vacuolated cells.

Furthermore, basophilic and periodic acid–Schiff positive colloidal aggregations occur in some adenohypophysial follicles as do epithelium-lined cavities. No distinct acidophils were observed. After applying the periodic acid–Schiff it was shown that the basophils contained periodic acid–Schiff positive material as in higher vertebrates. Some cells also stained after applying the Gomori aldehyde–fuchsin technique. It seems highly probable then that the pituitary of *Myxine* secretes carbohydrate-containing hormones in a similar manner to other vertebrates.

These observations, which will be reported in full elsewhere, indicate that the pituitary of *Myxine glutinosa* is possibly the simplest form of vertebrate pituitary and is a form which is not degenerate but is one in which the adenohypophysis has not become histologically differentiated.

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Swimming in Spider Crabs of the Genus *Macropodia*

The only adult Brachyura which have previously been recorded swimming are the Portunidae, which do so by rapid paddling of the last pair of peræopods. These are adapted for swimming by having the distal segments flattened, with the edges densely fringed with hairs.

Swimming has now also been observed in *Macropodia rostrata* (L.) and *Macropodia longirostris* (Fabricius), members of the Maiidae (spider crabs). Both these species occur sublittorally in the Isle of Man. The genus has very long legs which bear a number of long hairs, and swimming involves the four pairs of walking legs and the chelæ. All the limbs beat at the same rate, but not in phase, and are kept continually moving. At the start of the stroke they are raised in the flexed position, and are extended for the more rapid downbeat. Swimming may reach a speed of 5 cm. per sec. in a specimen of *Macropodia rostrata* with a carapace-length of 13 mm.

The pattern of movement of the legs has been followed, and it is the same in both species. The chelæ and walking legs will be referred to as *c*, 1, 2, 3, 4 respectively, on the right (*R*) and left (*L*) sides.

The swimming limbs can be divided into three groups: (1) L^3 and R^3 beat in phase with each other, but out of phase with all the other legs; that is, when group 1 is beating down, groups 2 and 3 are on the up-beat. (2) R^c, R^1, L^2 and L^4 beat as a unit, with L^4 slightly after the rest. (3) L^c, L^1, R^2 and R^4 also beat as a unit, with R^4 just behind the others. One of groups 2 and 3 starts to beat slightly before the other, and either may take the lead during a period of swimming. The rate of beating varies from once to twice per sec. The body of the crab is horizontal during swimming, and the sequence of movement of the legs prevents rolling or pitching. This is because the legs beating down at any moment are symmetrically arranged around the centre of gravity of the crab (for example, L^2 opposite R^2, and R^c and R^1 opposite L^3 and L^4).

In the laboratory, swimming may occur apparently spontaneously, as a result of attack by another crab, or on repeated disturbance. It occurs more readily in smaller specimens, and large ones can seldom be induced to swim.

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A New Cercaria of the Subfamily Gymnophallinae (Trematoda: Digenea) developing in a unique 'Parthenita' in *Littorina saxatilis* (Olivi)

A 'parthenita' containing furcocerous and tailless stages of a gymnophalline cercaria was found in the hæmolymph spaces of the digestive gland, gonad and gonoduct of the gastropod *Littorina saxatilis*. The unique feature of this 'parthenita' is that it has the same essential structure as the cercariæ which it produces. It is not yet possible to decide whether this 'parthenita' is, in fact, a cercaria possessing the kind of multiplication more usually associated with a redia or sporocyst; or whether it is a sporocyst or redia with a structure similar to a cercaria. Until this is decided the word 'parthenita' will be used here to indicate an analogy with the more usual redia or sporocyst found in digenetic trematodes.

This is the first record of a gymnophalline cercaria developing in a gastropod mollusc; previous records having been recorded from lamellibranchs. A detailed account of the information available to date on this species is being prepared for publication.

The 'parthenita' was found in 490 of 14,000 specimens of *Littorina saxatilis* examined between September 1958 and September 1959 at Aberystwyth and in 59 of 1,100 snails in August 1959 at Plymouth. Infection was almost exclusively confined to small molluscs, measuring from 0·6 to 5·0 mm. in length, occurring in the supralittoral zone; in favourable circumstances up to 40 per cent infection exists.

The fully formed 'parthenita' (Fig. 1) is spherical in shape, measuring 0·2–1·2 mm. in diameter and containing 20–2,000 tailless cercariæ. 5–70 fully formed 'parthenitæ' may occur in any one host. The whole surface of the 'parthenita' is covered with alternating rows of large backwardly directed spines. An oral sucker is present and specimens measuring less than 0·2 mm. in diameter have, also, a ventral sucker. The bifurcated alimentary canal consists of pharynx, œsophagus and two dilated cæca which are connected to the body-wall of the 'parthenita' by strands of tissue. There is no birth pore. The excretory system is best seen in specimens less than 0·3 mm. in diameter; in larger specimens the arrangement becomes difficult to observe. The translucent excretory vesicle is Y-shaped, the arms of the Y extending forwards as far as the posterior border of

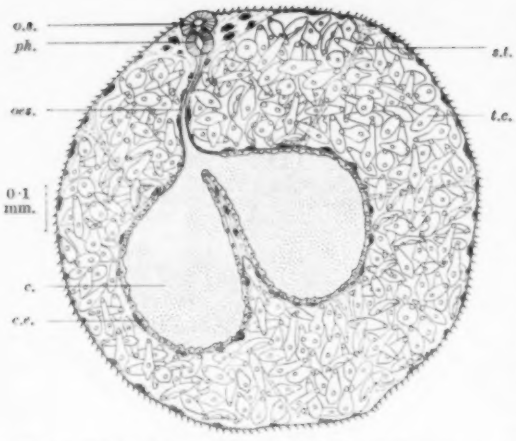

Fig. 1. A fully formed 'parthenita' containing tailless gymnophalline cercaria from *Littorina saxatilis*. *c*, Cæcum; *c.e.*, ciliated epithelial cells of cæcum; *oes.*, œsophagus; *o.s.*, oral sucker; *ph.*, pharynx; *s.t.*, tissue strand supporting cæcum; *t.c.*, tailless cercaria

the pharynx, curving inwards as they do so. The flame cell formula is $2((2 + 2) + (2)) = 12$. The 'parthenita', therefore, closely resembles a tailless gymnophalline cercaria.

Many 'parthenitæ' have been examined, and from these it has been possible to construct a series in development terminating in the formation of the fully formed 'parthenita' containing tailless cercariæ.

The earliest stage so far discovered is the mother 'parthenita', several generations of which may occur. The mother 'parthenita' measures 0·16–0·4 mm. in diameter and has all the anatomical features of the fully formed 'parthenita' except that it contains 5–12 developing 'parthenitæ' instead of cercariæ.

The contained 'parthenitæ' undergo a most unusual development, passing through a furcocercous stage similar in size and structure to the furcocercous cercariæ, which later develop into tailless cercariæ in the fully formed 'parthenita'. Unlike the furcocercous cercariæ, however, the tails are spineless and immobile; these furcocercous stages cannot swim when released artificially. During the course of further development the tail of each furcocercous stage degenerates and a cavity containing germinal balls is formed in the body. This results in the formation of a number of contractile tailless 'parthenitæ' with the characteristic features of a tailless gymnophalline cercaria but possessing, also, a cavity filled with germinal balls. These 'parthenitæ' increase in size and the pressure so produced causes the wall of the mother 'parthenita' to burst, releasing all the 'parthenitæ', which are by this time at a similar stage in development. The body of the liberated 'parthenita' measures 0·12–0·4 mm. in length, depending on the state of contraction. It is extremely mobile; moving through the hæmolymph spaces of the host by alternate attachment of oral and ventral suckers. The 'parthenitæ' gradually develop into immobile spheres measuring up to 1·2 mm. in diameter; the ventral sucker degenerates in specimens more than 0·2 mm. in diameter. This is the stage already described as the fully formed 'parthenita' containing the tailless cercariæ.

The development of the cercaria within the 'parthenita' shows a fundamental similarity to the development of the 'parthenita' itself. On release from the mother 'parthenita' the 'parthenita' contains a number of germinal balls. These first develop into furcocercous cercariæ with opaque mobile bodies measuring 0·12 mm. in length; the tail stem measures 0·042 mm. in length and the furcæ 0·043 mm. Both the body and the tail are covered with alternating rows of small spines. No eyespots or penetration glands are present. The excretory vesicle is Y-shaped and the flame cell formula is $2((1) + (1)) = 4$. If released artificially the furcocerous cercaria swims by lashing its tail but it survives only for a short time in these circumstances. While still inside the 'parthenita' the tails degenerate and extremely contractile transparent tailless cercariæ result. The following characters of these tailless cercariæ demonstrate how they differ from all previously described gymnophalline tailless cercariæ and metacercariæ; they indicate, also, how closely the tailless cercariæ resemble the 'parthenita' which produces them. They are extremely small, the body measuring 0·05–0·14 mm. in length according to the degree of contraction. Alternating rows of spines cover the whole body except for a region on the ventral surface between the oral and ventral suckers. The suckers are subequal. Three pairs of penetration glands are present, the posterior border of the largest reaching as far as the bifurcation of the gut. The arms of the translucent Y-shaped excretory vesicle are unbranched and extend forward as far as the posterior border of the pharynx, converging on it as they do so. The flame cell formula is $2((2 + 2) + (2)) = 12$. Rudiments of the genitalia lie behind the ventral sucker.

The formation of the tailless cercariæ within the fully formed 'parthenita' probably terminates the cycle of development in the molluscan host, the tailless stage being infective to the definitive host.

All previously recorded gymnophalline cercariæ develop in sporocysts in lamellibranchs. The metacercariæ, also, occur in lamellibranchs, with two exceptions which have been recorded from gastropods. Since the 'parthenita' and cercaria described here are almost identical in structure and development it is possible that this is a case in which the type of multiplication that usually occurs in the sporocyst has extended into the cercaria. In other words the germinal cells in the cercaria, instead of giving rise to the reproductive organs of the adult, remain germinal and continue to multiply by polyembryony with the result that the cercaria in effect becomes a parthenita. If this life-cycle includes, also, a more 'normal' sporocyst not yet found and preceding the above described 'parthenita' then this may be the case, the life-cycle then being a modification of that normally found in the Gymnophallinae. If, on the other hand, no such sporocyst or its equivalent is present then the life-cycle might be more primitive than that of any other known digenean, the 'parthenita' indicating how multiplication in the molluscan host could have developed from an adult. Speculations, however, are unwise until the entire life-cycle of this form is known. Attempts at solving the life-cycle experimentally are in progress.

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Forecasting the Annual Yield in Sea Fisheries

It is well known that the annual yield of the sea fisheries varies very much from year to year. An example of such variation is shown in Fig. 1 in which the broken curve shows the number (in millions) of skrei—spawning cod (*Gadus callarias*)—caught in the Lofoten fishing area of northern Norway in the years 1885–1958. It is obvious that such fluctuations make the planning of the fishing industry very difficult and that reliable forecasts would be helpful, even if they are only rough approximations.

In an earlier paper[1] I made the suggestion that it might be possible to compute yield forecasts by means of an aggregate of sine functions, using the periodic or period-like components found in series of measurements of the width of the annual growth zones in forest trees. The underlying idea was that the width of the growth zones and the size (in number) of the year-classes of fish populations might be determined by common factors. A rough outline of a theory was given.

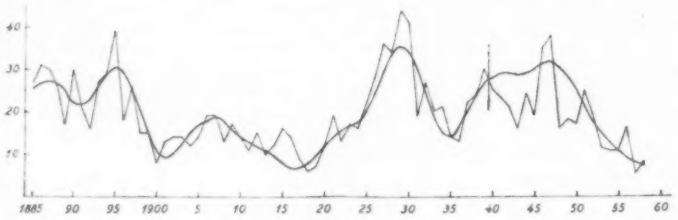

Fig. 1

Hjort[2] succeeded in showing that there are great fluctuations in the size of the year-classes in fish populations and concluded that these fluctuations were the main cause of the fluctuations in the yield. But fluctuations in the yield are also caused by other factors, such as weather conditions and altered fishing technique. Therefore, a successful forecast of the relative size of the stock of fish may not be a successful forecast of the yield; but it might show an approximately correct trend.

In 1939 I became acquainted with the results of the analyses of some series of measurements of the width of the growth zones of the Scots pine (*Pinus silvestris* L.) found by Ording[3]. His samples were taken in a forest in the neighbourhood of the Lofoten fishing area. Then, in order to verify my idea I fitted the series of annual yields of the skrei fisheries in the years 1885–1939 by a function constructed by means of four components (substituted by sine functions) found by Ording. The lengths of these components were 11, 17·5, 23 and 57 years. Also the phases of the components were taken from the results obtained by Ording. The actual yields were used merely for the computations by the least-square method of the weights of the components. The result, as well as the method of computation, is described in ref. 1. Later on I also included the 8·5 years component. The result so obtained is given by the smooth curve for the years 1885–1939 in Fig. 1.

Since this curve is constructed by means of an aggregate of sine functions, it can be extended to future years and might be used as a forecast of the annual yields. Actually, it is intended to be a forecast of the relative size of the stock of skrei.

The thin curve 1940–58 shows the actual yields and the thick curve the 1939 forecasts for the same years.

It is evident that differences of the same magnitude as those observed in the years 1885–1939 should be expected. The largest differences between actual yield and forecast are found for the war years 1941–45 and in the years 1948–49. In the fishery reports from the Lofoten area I have found nothing which shows conclusively that the fishing was hampered by the war. But, bearing in mind the changes in the general conditions brought about by the war, I think we can assume that this was in fact the case. In 1948 and 1949 the weather conditions were uncommonly unfavourable. Bearing this in mind, the following conclusions seem to be justified: (1) That the width of the growth-zones of the pine and the size of the year-classes of the skrei are determined by the same main factors. (2) That it is possible to compute reliable forecasts of the yield of fisheries by means of the components found in series of measurements of the width of the growth-zones of the pine.

There is no doubt that the technique of forecasting can be improved. But in order to make improvements possible, it is necessary to sample new series of measurements of the width of growth-zones in forest trees and try to get better estimates of the lengths and the phases of the more important components.

The same method might prove to give satisfactory forecasts in other fisheries, too. However, the fishing technique used in the Lofoten area is very conservative, so that it can be assumed that the yield is a fairly constant percentage of the stock of fish. In other fisheries this may not be so. It is obvious that, for example, the introduction of a new technique may cause disturbances which are difficult to overcome.

I am very much indebted to Gunnar Rollefsen, director of the Norwegian Sea Fishery Investigations, for supplying me with data.

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ANATOMY

Fine Innervation of the Carotid Body of the Rhesus Monkey

Although first mentioned in 1744[1], the first clear and accurate description of the carotid body was not published until 1862[2]. However, modern knowledge of the structure of this organ dates from 1926[3]. Since that time detailed macroscopic and microscopic studies of the structures found in the carotid bifurcation have been made on many different animal species. But in the Anthropoidea, except for the work of Sato[4], only macroscopic studies of this region have so far been undertaken (1852[5], 1899[6], 1928[7], 1932[8], 1935[9,10]). Sato's work is concerned only

with the general histology of the organ and not its detailed innervation.

This is a preliminary report of part of an investigation into the histology and cytology of the carotid body and carotid sinus of *Macaccus rhesus*.

Rhesus monkeys were anæsthetized and then perfused through the heart first with warm normal saline to wash out the blood, and then either with 10 per cent formal–saline or Bouin's fixative.

The carotid bifurcation and adjacent tissues and structures were carefully dissected out and either treated: (1) according to the Bielschowsky method for pieces with pyridine treatment[11], or (2) 7μ serial wax sections were subjected to Bodian's silver protargol method using silver proteinate obtained from Roque's Laboratory, Paris[12].

With both techniques, the only glomus cells found to be innervated were the chief cells (De Kock's Type 1 cells[13]). Many fine nerve fibres wind round the large individual chief cells, or groups of chief cells, either in a regular manner (Fig. 1) or to form a tangled meshwork (Fig. 2). The fine nerve fibres appear to be in direct contact with the chief cells with no interposed interstitial cells[13–15]. No evidence was found of De Castro's "menisque terminaux"[16] within the cytoplasm of the cells.

The fine nerve terminals are of the order of 0·2–0·5μ in size. They fuse with each other to form larger fibres (Fig. 3) which run between the glomeruli of chief cells, and, joining other large fibres, emerge from the carotid body to form its contribution to the sinus nerve.

No evidence was found to support the observation[17] that the heavily medullated fibres of the sinus nerve, which most authorities consider as innervating both carotid body and sinus, do not take part in the innervation of the carotid body but are merely passing through on their way to the carotid sinus.

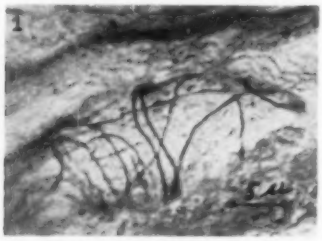

Fig. 1. A group of glomus cells showing the fairly regular arrangement of their innervating nerve fibres

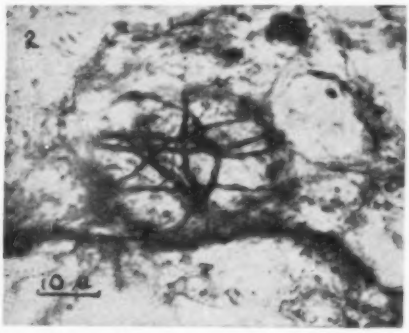

Fig. 2. Glomus cells enclosed in a tangled meshwork of nerve fibres

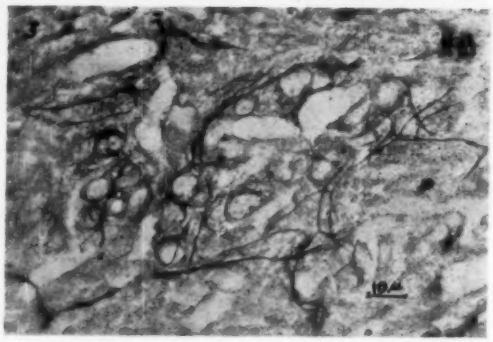

Fig. 3. Part of the carotid body showing the large globular glomus cells, sinusoids, and large nerve fibres running between the glomeruli

One of the difficulties encountered in this study was that the smallest nerve fibres are very near in size to the limits of resolution of the light microscope. However, further support for these observations that: (1) only the Type I glomus cells are directly innervated by the glossopharyngeal fibres without the intervention of interstitial cells, and (2) that no evidence could be found for an innervation of De Kock's Type II cells, comes from the recently made electron microscope studies on the carotid body of the cat by Garner and Duncan[18].

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HÆMATOLOGY

Some Red-Cell Antigens of *Bison bonasus* as compared to Red-Cell Antigens of Domestic Cattle

Red cells of a bison (*Bison bonasus*) were submitted to serological examination in order to find antigenic similarity and differences between the erythrocytes of bison and those of domestic cattle. The blood groups phenotype of the individual in study was as follows:

$$A - A', E'_1 - W, X_2 - F - J - L$$

(The phenotype was determined using 38 bovine blood-typing reagents:

A; B, G, I, E_1, E_2, O_1, O_3, Q, T_1, Y, A′, D′, E'_1, E'_3, J′, I′, K′; C_1, C_2, X_1, X_2, X_3, W, L′; F, V; J; L; M; S_1, S_2, U_1, U_2, U′; Z, ZZ; Z′.

These reagents were kindly given to us by the Department of Genetics, University of Wisconsin, and by the Institute of Animal Breeding, Royal Agricultural College, Uppsala.)

The red cells of the bison studied were injected into seven rabbits and two heads of cattle (twins). The blood group phenotypes of the immunized animals were as follows:

No. 20/57 — I, Y, E'_3, K′ — C_2, X_2 — F — S_2

No. 21/57 — I, Y, E'_3, K′ — X_2 — F

(For determination of these phenotypes 19 standard reagents—A; B, G, I, Y, K′; C_2X_2, L′; F, V; J; L; S_1, S_2, U_1, U_2; Z—were used.)

After one series of immunizations (10 injections into each rabbit and three injections into each of the cattle) the animals were bled. The rabbit-immune sera reacted in high titres with both bison and cattle red cells (Table 1). The two cattle-immune sera exhibited the lytic titres of 1 : 64 and 1 : 16 against the bison red cells. These sera contained also antibodies for some samples of cattle red cells.

Table 1. Results of the Agglutination and Lysis Tests, using Unabsorbed and Absorbed Rabbit Anti-bison Red-Cell Sera with Bison and Cattle Red Cells

Serum		Titres of red cells			
		Bison		Cattle	
		Agglutination	Lysis	Agglutination	Lysis
No. 7272	Unabsorbed	2,048	4,096	0	1,024
	Absorbed	32	64	0	0
No. 7144	Unabsorbed	2,048	2,048	0	512
	Absorbed	16	16	0	0
Pooled serum of all 7 rabbits	Unabsorbed	8,192	8,192	0	1,024
	Absorbed	64	128	0	0

Serum No. 20/57 reacted with 20 of the 110 tested cattle red-cell samples; serum No. 21/57 reacted with 45 of the 109 tested samples. The specificity of these antibodies could not be identified with any of the 38 standard reagents; more detailed studies on this problem are in progress.

In order to test whether the bison red cells bear antigenic substances particular only to this species, the rabbit anti-bison red cell immune sera were submitted to absorption with cattle red cells. It was assumed that this absorption would remove antibodies for those bison antigens which are identical or very similar to some of the cattle red-cell antigens.

The 10 samples of the cattle red cells employed in the absorption contained all known antigens except the antigens: H, I, P, E_2 and Z'. In the given case, only the absence of H and P antigens was of consequence. The remaining antigens, that is, I, E_2 and Z', were not present in the bison blood sample, and therefore there was no possibility that any antibodies could be formed against these antigens.

The rabbit anti-bison red-cell sera absorbed with cattle red cells gave a negative reaction with each of the 48 cattle red-cell samples, whereas they gave a clearly positive reaction with the red cells of the bison, and what is more, even in rather high dilutions. These results are presented in Table 1.

Despite the fact that these tests require further development and repetition on a wider range of specimens, our results allow us to conclude that:

(1) The erythrocytes of bison blood contain the antigens: A of system A, $A'E'_1$ of system B, WX_2 of system C, F of system FV, J^{sc} of system J, L of system L. They are all either identical or very similar to the antigens present in the domestic cattle.

(2) The red cells of the tested bison contain other, still unidentified antigenic factor(s) which may perhaps be found also in some of the cattle red-cell samples; these factors are antigenic to those individuals of cattle which do not contain them.

(3) The red cells of the tested bison contain one or more antigenic properties specific for them, which were not found in the cattle red-cell samples studied

Thanks are due to Profs. M. R. Irwin and W. H. Stone of the University of Wisconsin for their friendly encouragement of this research and personal suggestions, to Dr. S. Dubiski of the Institute of Hæmatology, Warsaw, for valuable advice and comments, and to S. Wezyk for handling part of the field work.

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Haptoglobin Types in South-East Asia

The discovery that the haptoglobin present in human serum can be resolved into a number of different types by electrophoresis in starch gel[1] and the subsequent demonstration that these types are controlled genetically[2] has opened a new field of investigation in human population genetics. Already the distribution of the haptoglobin phenotypes has been reported for a number of different populations in the world[3], and several other investigations privately reported to us are in progress.

The present study covers the distribution of the haptoglobin types among the three major ethnic groups in Malaya. Blood samples were obtained by venepuncture from healthy adults. In Perlis the samples were collected into Bayer 'venules' and sent to Perth as clotted blood: in Kuala Lumpur the samples were collected into syringes, and the serum was separated from the clot before being sent to Perth. In both cases the samples were flown in ice-cold containers and were generally in Perth within 48 hr. from the time of collection.

During the early part of the investigation electrophoresis was carried out in horizontal trays according to the original method of Smithies[4], using the hydrolysed starch supplied by the Connaught Medical Research Laboratories and borate buffer. Hæmoglobin was added to the samples prior to electrophoresis to give a concentration of approx-

Table 1. Number of Persons by Haptoglobin Type in various South-East Asian Populations

Place	Ethnic Group	*Hp* type					Total
		0–0	1–1	2–1	2–1 (mod)	2–2	
Perlis	Malays	0	5	53	0	99	157
	Indians	1	2	17	2	110	132
Kuala Lumpur	Malays	2	7	31	1	38	79
	Chinese	1	9	36	0	55	101
	Indians	3	2	13	0	69	87
Perth (students)	Chinese	1	9	21	—	35	66

Table 2

Ethnic group	No. tested	Distribution (per cent)					Gene frequency		$\chi^2_{(1)}$	P
		0–0	1–1	2–1	2–1 (mod.)	2–2	Hp^1	Hp^2		
Malays	236	0·8	5·1	35·6	0·4	58·1	0·24	0·76	0·2	0·7–0·5
Chinese	167	1·2	10·8	34·1	—	53·9	0·28	0·72	3·58	0·1–0·05
Indians	219	1·8	1·8	13·7	0·9	81·7	0·09	0·91	0·70	0·5–0·3

imately 100 mgm. hæmoglobin/100 ml. of serum. The haptoglobin bands were rendered visible by means of a benzidine reagent. In the latter part of the investigation electrophoresis was carried out in vertical trays[5], the other conditions remaining the same.

Table 1 records the haptoglobin types for the Perlis and Kuala Lumpur samples together with a smaller number of samples collected from Asian students in Perth. There is no significant heterogeneity for any of the ethnic groups with respect to the place from which the samples came. For the calculation of gene frequencies the samples have been combined and the 0–0 and *Hp* 2–1 (mod) phenotypes excluded from the calculation.

Several points of interest arise when the present results, as shown in Table 2, are compared with those reported for other populations. In this material the modified *Hp* 2–1 phenotype[6] is rare, and no cases were noted among the Chinese, only two cases in Indians, and a single case in Malays. Similarly, individuals completely lacking haptoglobin (*Hp* 0–0) are infrequent also, though more common than in a control population of Australian whites[7]. It is extremely difficult to distinguish the *Hp* 0–0 phenotype with certainty. In many cases the amount of haptoglobin present in *Hp* 2–2 individuals is so slight that it can readily escape detection. All cases where haptoglobin was missing were examined at least three times on successive days. But some of the *Hp* 0–0 individuals may still represent *Hp* 2–2 individuals with haptoglobin concentrations below the level necessary for detection. Other cases may be individuals temporarily lacking haptoglobin due to hæmolytic episodes in their past history[8].

It has been suggested that the *Hp* 0–0 phenotype may be due to a modifying gene at a separate locus[9], or alternatively represents the homozygous condition for the Hp^{2M} gene (Giblett, E. R., personal communications). We do not have family material which would help in the interpretation of this phenomenon, but in the latter case since the modified *Hp* 2–1 type is infrequent in our series it would suggest that the homozygous condition Hp^{2M}/Hp^{2M} should be rare in these three ethnic groups.

The chief point which emerges from the present study is the high frequency of the Hp^2 gene in this area, reaching its highest reported value in Indians. This result is in agreement with the value reported by Sutton for a group of Asiatic Indian students in North America[10]. Further studies on populations in the Near and Middle East and in central Asia will be necessary before a clear picture of the *Hp* gene distribution in Asia as a whole is obtained. We are extending our own investigations to cover the aboriginal populations in this area, and a survey of the variations in the *Tf* and *Gm* serum protein groups for all these populations in South-East Asia is at present in progress.

Our thanks are due to the Australian National Health and Medical Research Council and the Research Grants Committee of the University of Western Australia, for financial assistance, and to Dr. Eloise Giblett for a sample of a *Hp* 2–1 (mod) serum.

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Retention of Potassium by Human Erythrocyte Ghosts

When ghosts prepared from human erythrocytes by lysis in water are washed in a saline medium, sodium and potassium ions are nearly uniformly distributed across the ghost membranes[1]. In attempting to prepare ghosts with higher potassium and lower sodium concentrations than those of a saline medium we have found that incubation at 37° C. enables them partially to retain potassium and keep out sodium during subsequent washings and incubation.

A hæmolysate of completely lysed human erythrocytes was made approximately isotonic with plasma by the addition of 3 *M* salt solution (5 parts potassium chloride and 1 part sodium chloride) to give final concentrations of 150 m*M* potassium chloride and 30 m*M* sodium chloride. The ghosts were washed three times at room temperature in a saline medium (0·17 *M* sodium chloride, 0·005 *M* potassium chloride, 0·01 *M* sodium phosphate, *p*H 7·4), this process taking about 30 min. The potassium concentration in the ghosts (hæmoglobin content about 10 per cent of original erythrocytes) was approximately three times that in the medium, and incubation of the hæmolysate at various temperatures and for different periods of time before the addition of 3 *M* salt solution made no difference. However, when the suspension was incubated for about 30 min. at 37° C. in 150 m*M* potassium chloride after the addition of

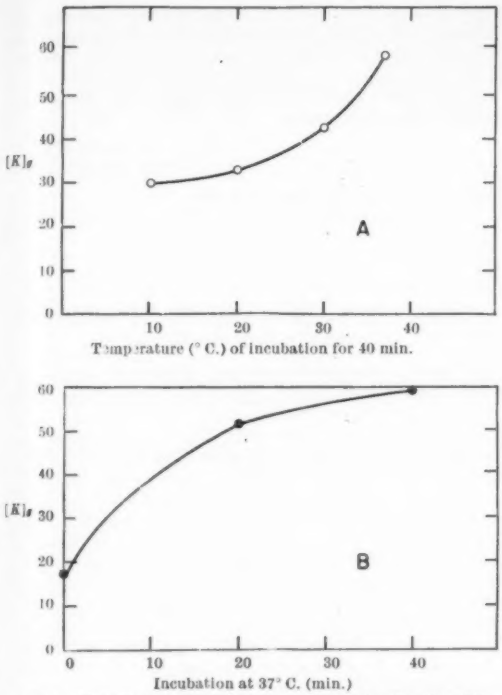

Fig. 1. The effect of incubation of a hæmolysate after the addition of 3 *M* salt solution to give 150 m*M* potassium chloride and 30 m*M* sodium chloride, at different temperatures for 40 min. (graph *A*), and at 37° C. for varying periods of time (graph *B*), on the potassium concentration of red cell ghosts afterwards washed three times in physiological saline solution. $[K]_g$ has the unit: m.moles potassium/litre of ghosts

the 3 *M* salt solution, the washed ghosts then contained about twelve times more potassium than the medium. Both the temperature and the period of incubation affected the amount of potassium found in the washed ghosts (Fig. 1). The more potassium present the higher the temperature of incubation (range 10–37° C.), and ghosts incubated for 40 min. at 10° C. contained twice as much potassium as those unincubated at any temperature.

Ghosts spun down from a hæmolysate and then incubated in 0·17 *M* potassium chloride acquired a similar ability to retain potassium, showing that the non-particulate part of the hæmolysate was not required. The final potassium concentration of washed ghosts, however prepared, was never more than 50 per cent of the concentration in the suspension before washing. This may be because half the ghosts were equilibrated with the washing medium or because all the ghosts retained only half the maximum possible potassium, although these explanations are not exclusive. During the incubation of washed ghosts in the saline medium for 4 hr. at 37° C., about 5 m.moles potassium/l. ghosts/hr. were lost, which is about twice the rate of loss of potassium from fresh starved erythrocytes.

The results show that ghosts containing about 10 per cent of the hæmoglobin of erythrocytes may partially retain potassium during three washes and subsequent incubation in a saline medium, suggesting that after osmotic hæmolysis ghosts may regain some degree of the low permeability to potassium which is characteristic of erythrocytes.

A portion of this work was performed during tenure of the following fellowships: (J. F. H.) Research Fellow, U.S. Public Health Service; (D. C. T.) Fellow, Foundation for Infantile Paralysis; (R. W.) Beit Memorial Research Fellow.

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Porphyrin Biosynthesis in the Leucocyte

The presence, in various animal tissues not primarily hæmatopoietic, of some or all of the enzyme systems taking part in porphyrin and hæm biosynthesis has been reported by many authors. However, until recently, any investigation of porphyrin biosynthesis in the leucocyte has been hindered by the contamination of tissue preparations with erythrocytes having a much greater synthetic capacity. During a systematic study of several enzyme systems in the normal and pathological leucocyte now being carried out in this clinic, a method of preparing undamaged leucocytes, completely free of erythrocytes and thrombocytes, has been developed[1]. It therefore seemed interesting to study the behaviour of these enzyme systems in this readily available and easily quantitated tissue.

After incubation of a homogenate of normal human leucocytes with δ-amino-lævulinic acid under standard conditions (4 hr. at 37° C., *p*H 7·4, 0·8 × 10^7 leucocytes/400 μl.), a substance giving a red colour insoluble in chloroform on reaction with *p*-dimethylaminobenzaldehyde is found in the deproteinized supernatant. The absorption spectra of this colour and that formed by a solution of porphobilinogen in the incubation medium are exactly superposable between 450 and 600 mμ, with peaks at 522 and 555 mμ. No fluorescence is seen under Woods light and only a very slight peak between 400 and 410 mμ. After heating the deproteinized supernatant at 100° C., and *p*H 1–2, for ½ hr., a distinct red fluorescence is seen, together with the appearance of a peak at 407 mμ, indicating strongly that the Ehrlich reacting substance present was porphobilinogen, transformable to porphyrin. A quantitative analysis of these results will be published elsewhere.

It is interesting to note that leucocytes prepared from the blood of normal guinea pigs showed synthesis of porphyrin after 4 hr. incubation. Due to the small amounts no analysis or purification of any porphyrins formed has been attempted. The concentrations were roughly compared by comparison of the corrected absorption at the Soret peak, that is, of the deproteinized incubate supernatants.

Chromatography of the supernatant of an incubate of authentic porphobilinogen with normal leucocytes under standard conditions showed two spots, one corresponding to the porphobilinogen marker together with another, remaining near the origin and becoming blue on standing. Attempts at chromatography of the supernatant from an incubation with δ-amino-

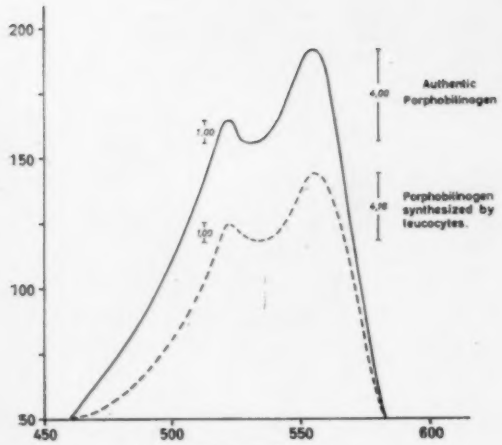

Fig. 1

lævulinic acid were not reliable due to the contamination of the minute quantities involved. However, while no typical porphobilinogen spot was seen, one remaining near the origin and turning blue on standing was present. Demonstration either that this spot is similar to the atypical spot on the porphobilinogen chromatograms or is only an artefact must wait until very much larger quantities are available.

If the incubation is continued, porphyrin fluorescence is seen in the supernatant after 24 hr., indicating that its absence earlier in the incubation could be a problem of the concentration of the different factors involved. However, incubation of a more concentrated homogenate, approx. 40×10^6 leucocytes/incubation, led to synthesis of proportionately greater quantities of porphobilinogen, always without the appearance of porphyrin. Incubation of authentic porphobilinogen in physiological quantity (6 mμM) with leucocytes under standard conditions leads to porphyrin formation apparently in excess of that in the control without leucocytes; but as this remains constant when either the concentration of porphobilinogen or of leucocytes is increased, the apparent difference is probably due to the method of estimation. It seems most likely that the presence of porphyrin after 24 hr. incubation is due to the spontaneous condensation of the porphobilinogen synthesized, and that the mature leucocyte, while able to form porphobilinogen, cannot carry the synthesis any further. There are two possible explanations of this: that porphobilinogen-deaminase is in fact absent, or that it is present but inhibited in some fashion.

That it is present and active in the immature cell is indicated by the results of incubation of leucocytes from certain cases of leukæmia. When the cell preparation contained a large proportion of young cells, either monoblasts, lymphoblasts or myeloblasts, there was net synthesis of porphyrin after incubation with δ-amino-lævulinic acid under standard conditions. It is possible that, on maturation of the leucocyte, a system which slightly transforms porphobilinogen so that it cannot be utilized by porphobilinogen-deaminase may come into play.

It is also interesting to note that in two cases, one of acute myeloid and one of acute lymphatic leukæmia where the cells contained little or no protoplasm, there was no synthesis of either porphobilinogen or porphyrin from δ-amino-lævulinic acid, demonstrating that these enzymes are probably exclusively present in the cytoplasm.

We wish to thank Prof. C. Rimington for his advice on our chromatograms.

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Hæmolytic Disease of the Newborn probably due to Anti-f

A case is briefly presented, the chief feature of which is the production of anti-f held to be responsible for severe hæmolytic disease of the newborn. Prior to her marriage the patient, Mrs. M. H., group O, Rh positive, most likely genotype R^1R^1 (DCe/DCe), was transfused with Rh positive blood of unknown genotype. The first pregnancy ended in a full-term stillbirth but no autopsy was performed. The second pregnancy also terminated in a full-term stillborn due to hydrops. During this second delivery the patient received 500 c.c. of group O, Rh negative blood followed by a severe hæmolytic reaction and renal shutdown. The patient recovered with the aid of an artificial kidney and received a total of 13 pints of blood—all compatible by an indirect Coombs cross-match.

The third infant delivered in 1958 by Cæsarean section at 38 weeks had severe hæmolytic disease and recovered after replacement transfusion with group O, Rh positive compatible blood.

The first blood specimen of Mrs. M. H. was submitted during August 1959 in the seventh month of her fourth pregnancy. Screening tests indicated that the serum contained an antibody which reacted with albumin-suspended cells. On testing the serum with a panel of cells it was observed that bloods R^1R^1(DCe/DCe), R^1R^2(DCe/DcE), $r'r'$(dCe/dCe) and $r''r''$(dCE/dCE) failed to react, while R^2R^2(DcE/DcE) gave either negative or very weak reactions. Cells of genotypes R^1r and rr and others having antigens c and e carried by the same chromosome in single or double dose reacted strongly. The failure of the serum to react with the 4 $r'r'$ and $r''r''$ bloods excludes the presence of anti-d. These findings suggested the presence of a very weak anti-c but a predominating antibody, anti-f. In order to exclude a prozone indicative of a potent anti-c, the serum was titrated with R^2R^2 cells and this result confirmed the presence of a very weak anti-c. The titre of anti-f as determined by tests with rr bloods was 1 : 32. These findings were fully confirmed in tests of a second specimen of the patient's serum submitted later in the seventh month.

Mrs. M. H. was delivered by Cæsarean section one month prior to the expected date of delivery and the baby was reported as normal at birth, but soon developed jaundice. The direct Coombs test of the cord blood was moderately positive. The infant

Table 1. Tests of the Red Cells of the Parents and Affected Infant

		D	C	E	c	e	f	K	k	Fy^a	S	s	M	N	P
Father	O	+	+	0	+	+	+	0	+	0	0	+	0	+	+
Mother	A	+	+	0	0	+	0	+	+	+	+	0	+	0	+
Cord	A	+	+	0	+	+	+			Not tested					

Table 2

Probable genotype	No. of tests	Reactions with anti-f	Maternal serum		Cord serum		Eluate cord red cells	
			1	2	1	2	1	2
R^1R^1	3	0	0	0	0	0	0	0
R^1R^2	1	0	0	0	0	0	0	0
R^2R^2	4	0	0	0	0	0	0	0
R^2R^0	1	0	+	0	0	0	0	0
R^1r	2	+	++++	++++	++	++++	0	++++
R^2r	1	+	++++	++++	+++	++++	0	++++
R^0r	1	+	++++	++++	+++	++++	0	++++
$r'r$	2	+	++++	++++	+	++++	0	++++
$r''r$	2	+	++++	++++	++++	++++	0	++++
rr	4	+	++++	++++	++++	++++	0	++++
$r'r'$	4	0	0	0	0	0	nt	nt
$r'r''$	1	0	0	0	nt	nt	nt	nt
$r''r''$	1	0	0	0	0	0	nt	nt
R^{0z} $(D--/D--)$	4	nt	0	0	nt	nt	nt	nt

nt, not tested.

Column 1 indicates the reactions with albumin-suspended red cells after centrifugation; column 2 indicates the reactions with indirect antiglobulin test on the albumin-suspended red cells.

The anti-f and $r'r''$ bloods were supplied by courtesy of Dr. R. E. Rosenfield, Blood Bank, Mount Sinai Hospital, New York.

recovered following a replacement transfusion carried out with Rh positive blood, compatible with the mother's serum.

An eluate prepared from the cord red cells by the heat technique, revealed the presence of anti-f when tested with albumin-suspended cells followed by the indirect antiglobulin reaction. The results obtained in simultaneous tests of the maternal serum, cord serum and the eluate of the infant's coated red cells with a selected panel of test cells are given in Table 2.

Since the supply of maternal serum obtained at delivery was exhausted, the specimen taken at 7 months was compared with cord serum in parallel titrations with ficinated red cells. The results are given in Table 3.

Table 3

Test cell	Maternal serum	Cord serum	Test for
R^2R^2	1 : 4	1 : 4	anti-c
rr	1 : 16	1 : 32	anti-f

The titre for anti-c was less than the titre for anti-f, and this observation together with the findings of the cord cell eluates supports the view that anti-f induced the hæmolytic disease. This case is unique because the patient was expected to produce the more common anti-c and perhaps anti-f. Surprisingly, anti-c was very weak and a more potent and readily detectable anti-f was produced.

In the first example of anti-f, produced by a patient immunized by transfusions, there were additional antibodies (anti-N, anti-K and anti-S), but anti-f was the only Rh antibody produced[1,2]. Anti-f is frequently found as a secondary antibody in most anti-c and anti-e sera[3], but its demonstration is not a simple matter and requires absorption with selected cells and testing with enzyme-treated cells.

There is no apparent explanation as to why this mother who should have produced the more usual combination of a strong anti-c and perhaps some anti-f responded preferentially to the 'weaker' antigen. Studies show nothing unusual in the genotype of her husband's blood. His c antigen scored the same as other R^1r bloods. The antigenic stimulus in this case, however, was provided prior to her first pregnancy by transfusions of bloods which were probably f positive and during the delivery of the second stillbirth by Rh negative, and, therefore, f positive blood. There is in the literature at least one example of a patient immunized by pregnancy who produced the rare instead of the more common antibody[4]. In this case an Rh negative patient produced a more potent anti-C and a much weaker anti-D. Her husband was of the unusual genotype R^1r' (*DCe/dCe*) with suppression of D by gene interaction with the partner chromosome r' (*dCe*). We and others have also observed several examples of Rh negative women who produced anti-K or other antibodies and not anti-D even though their husbands were Rh positive.

We wish to thank Dr. Robert J. Collins, Snyder, New York, for submitting these specimens and supplying us with the case history.

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PATHOLOGY

Auto-Antibody in Rats with *Plasmodium berghei*

IN human malaria it has long been recognized that total loss of blood is significantly more extensive than loss which can be attributed to the direct rupture of parasites emerging from infected erythrocytes[1]. In a comparative survey of loss of blood and replacement in plasmodial infections of other mammals and birds, excessive erythrocyte destruction of the same type has been observed in all except one of the host–parasite combinations studied to date, namely in rats with *Plasmodium berghei*[2] and *Plasmodium vinckei*[3], in rhesus monkeys with *Plasmodium cynomolgi*, *Plasmodium gonderi* and *Plasmodium knowlesi*[4], and in chickens with *Plasmodium lophurae* (Zuckerman, A., unpublished results).

In this survey excessive loss was particularly striking in infections with mild or negligible para-

sitæmia. In such cases the main anæmic bout generally follows peak parasitæmia by at least a few days, and usually occurs when parasitæmia has become microscopically negligible or even latent. Since direct rupture due to emerging parasites cannot but be negligible when parasitæmia is so low; and while during the same period up to a third or even more of the circulating red cells may rapidly be destroyed, the results clearly show that some agency other than direct parasitic rupture is engaged in destroying red cells.

A working hypothesis to explain this excessive loss was suggested in the earlier papers of the survey, namely, that red cells may be sufficiently altered by the presence of parasites to become antigenic, and that they may then give rise to the production of auto-antibody.

The host–parasite combination of *P. berghei* in the adult rat is a convenient one for demonstrating excessive loss of blood, since parasitæmia is low and loss of blood high. Excessive loss thus stands out clearly, and is not masked by loss due to the parasitic rupture of host cells.

Auto-antibody was sought in such rats with the aid of a modified Coombs's test using anti-rat-serum rabbit serum as antiserum. The antiserum, as well as normal control rabbit serum, was exposed to normal washed rat erythrocytes before use, to remove an anti-rat-cell agglutinin regularly present in rabbit serum.

Forty-nine Coombs's tests done in 14 infected rats were positive during the period following parasitic crisis, while Coombs's negatives were regularly obtained from the same rats before crisis. To date, no infected rat which survived until latency has failed to pass through an evanescent period during which its red cells were antibody-coated. In no case did agglutination of Coombs's positive red cells occur in the presence of normal adsorbed rabbit serum. Forty-five tests done with the blood of eight uninfected control rats exposed to normal or anti-rat-serum rabbit serum were all Coombs's negative.

Antibody coating of erythrocytes, negative before crisis and positive after crisis, wanes and finally disappears as blood counts return to normal. The period of antibody coating thus closely corresponds to that of excessive loss of blood. Since latency commences at about the same time, the overwhelming majority of the coated cells must have been uninfected rather than parasitized.

Removal of the antibody-coated cells from the circulation is thought to be the cause of the excessive anæmia.

Bilirubin was sought in the serum of four rats at the height of the anæmic bout, with negative results. Antibody coating at this time is therefore not likely to have led to intravascular hæmolysis. It is more likely that coated cells, being opsonized, are filtered out of the circulation by elements of the lymphoid–macrophage system. It is germane that unparasitized red cells, together with infected cells, are avidly taken up at and after crisis by macrophages of monkeys infected with *Plasmodium brasilianum*[5], *P. cynomolgi* and *P. knowlesi*[6]; and that chicken macrophages *in vitro*, in the presence of blood infected with *P. gallinaceum* or *P. lophurae* suspended in homologous hyper-immune serum, ingest both normal and infected cells[7].

The above experiments are compatible with the view that auto-antibody against their own uninfected red cells is formed in mature rats infected with *P. berghei*. The fact that excessive loss of blood similar to that in *P. berghei* rats has also been observed in a broad spectrum of plasmodial infections in hosts of widely varying phylogeny suggests that a similar process may prove to be a general feature of malarial pathology.

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Anti-tumour Activities of Copper Chelates

Chelating agents have been shown to interfere with the utilization of metals by micro-organisms, robbing cells of their necessary metals[1]. Retardation of growth of a tumour (sarcoma 37) by the administration of two chelating agents, dipyridyl and O-phenanthroline, was observed by Leiter *et al.*[2]. Now, α-ketoglutaric acid oxime has been found to form its copper, iron, or nickel chelate at the physiological *p*H, and its antitumour activity *in vitro* was observed by the stained cell count method[3].

The immediate injurious action of the oxime (100 mgm./ml.) on the selective permeability of Ehrlich carcinoma cells was shown to be increased by the formation of chelate compounds. The same action of the chelates, copper and iron-α-ketoglutaric acid oxime, was brought about by a concentration of 25 mgm./ml. calculated as the oxime. To show the clear antitumour activity, the hydrophilic groups of the chelating agent should be masked by the metal ion to form the chelate compound which will be more permeable through cell membranes including the lipoid layer[4].

Dimethylglyoxime did not exhibit antitumour activities at a dose of 50 mgm./kgm./day *in vivo*. But the oxime (3–6 mgm./kgm./day), a chelating agent, exhibited potential antitumour activities on Ehrlich carcinoma and Crocker sarcoma 180 in the presence of copper, and not in the co-existence of other metal ions, such as nickel, cobalt, zinc, iron, manganese, magnesium and mercury. If antitumour activity of the chelate compound is only due to that permeable nature, the dimethylglyoxime should also exhibit the activity in the existence of other metals. But the activity was only exhibited by the copper chelate compound.

Not all the copper chelates always exhibit antitumour activity. The ligand moiety may also take a significant part in the activity. If the activity be exhibited by the non-ionic molecule of the copper chelates, copper-biacetyl-monoxime should also exhibit antitumour activity.

Both copper-dimethylglyoxime and copper-biacetylmonoxime inhibited the dehydrogenase activity of Ehrlich carcinoma cells at the same concentration of 25 γ/ml. But the former destroyed the selective permeability of tumour cells at 25 γ/ml.; nevertheless in the case of the latter chelate more than 180 γ/ml. were required to cause such action. As has already

been described, the former exhibited clear antitumour activity *in vivo*, while the latter did not. Both of them might permeate the cells to inhibit dehydrogenase activity, and only the former clearly inhibited tumour growth.

De Long *et al.*[5] observed a decreased calcium content which is peculiar to tumour tissues. Ambrose *et al.*[6] observed a marked difference of the electrophoretic motility between tumour and normal cells. As suggested by Brachet[4], if the increased motility of the cancer cells is to be attributed to different properties of the cell membrane, the antitumour substances, having specific inhibitory actions by breaking exclusively the selective permeability of cancer cells, may be formed. Some copper chelates having an adequate ligand moiety may exhibit the specific inhibitory action on tumour cells.

A full account of this work including the antitumour activities of the copper-dimethylglyoxime is to be published in the journal *Gann*.

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'Phenergan' and Versene in Dietary Liver Necrosis

RATS fed on Himsworth's[1] necrogenic diet have livers that are histologically undamaged, except for mild fatty infiltration, until an episode of acute massive necrosis occurs. Schwarz *et al.*[2] reported that liver slices from animals fed a necrogenic diet showed a metabolic abnormality at times when the livers were still normal histologically. The abnormality consists of an inability of these slices to maintain oxygen uptake for more than about 30 min. when incubated in Ringer solution at 38° with oxygen.

In the present investigation acute massive liver necrosis overtook the weanling rats after about fourteen days of feeding the yeast diet (mean 17·5, *S.D.* 4·0). Slices were taken from rats without liver necrosis at times after the seventh day of feeding the diet. Oxygen uptake was measured by the Warburg technique with Ringer phosphate[3] solution with 1·2 m*M* calcium as the incubation medium. Respiration declined to 50 per cent of initial rates in 40–60 min. This decline was invariably and completely prevented over test periods of 150 min. by the addition of 10^{-4} *M* 'Phenergan' (10-(2-dimethyl amino-*iso*propyl)phenothiazine hydrochloride) or 10^{-3} *M* versene (sodium ethylenediamine tetraacetate) (Fig. 1).

The initial rate of oxygen uptake in Ringer solution was the same as the rate maintained by the protective additions, and was similar to the rate of oxygen uptake by slices from control rats fed the necrogenic diet plus α-tocopherol (which completely prevents necrosis).

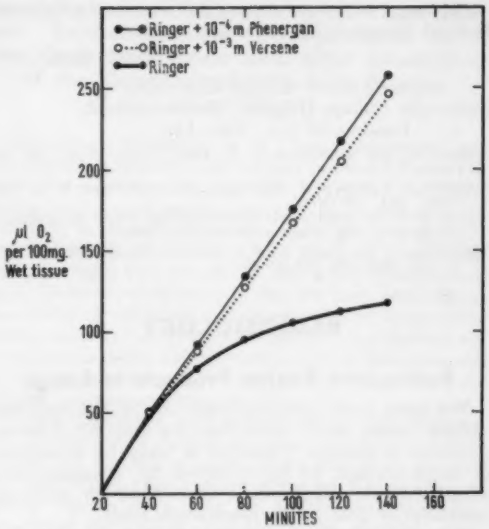

Fig. 1. Oxygen uptake of liver slices. Rat on yeast diet for 9 days

If 'Phenergan' is added to the yeast diet fed to the rats the onset of massive liver necrosis is delayed (Fig. 2).

The same effect is found if the two groups of rats are pair fed, and it is therefore not due to the decrease in food intake associated with the first days of feeding 'Phenergan'.

'Phenergan' has been found to prevent liver damage in thioacetamide poisoning[4]. A possible explanation for these effects in dietary liver necrosis is that both 'Phenergan' and versene act by blocking a chain of reactions where trauma leads to release of toxic breakdown products and further damage results. Such trauma would be provided by cutting the slices, or, *in vivo*, by absorption of toxic substances from the diet. Work is in progress to elucidate the nature of the biochemical lesion.

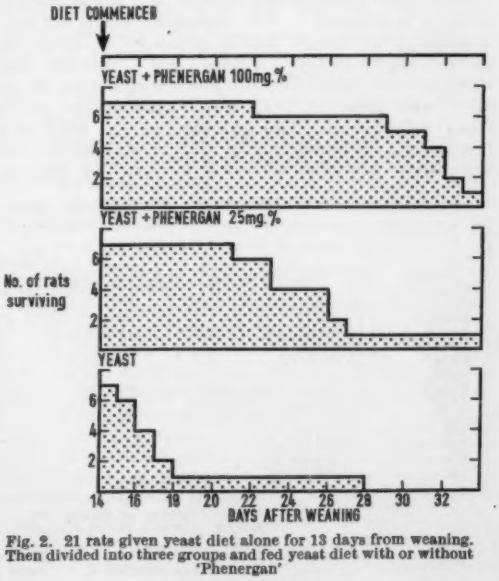

Fig. 2. 21 rats given yeast diet alone for 13 days from weaning. Then divided into three groups and fed yeast diet with or without 'Phenergan'

This work was done during the tenure of a Colonial Medical Research Scholarship.

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RADIOBIOLOGY

Radioactive Fission Products in Lungs

We have made measurements on the radioactivity of lung tissue, which show that the amount of fission products at present deposited in lungs by inhalation is large enough to be detected by comparatively simple means. This seems of interest for studying retention of dust in the respiratory tract.

Gamma-scintillation spectra of dust samples collected and measured in Vienna since July 1958 have all shown three characteristic photo-peaks: at (1) 145 keV., (2) 490 keV. and (3) 750 keV. This is in agreement with observations at other locations on top soil[1], rain-water[2], etc. Fig. 1*a* gives the spectrum of one of the dust samples collected in Vienna by filtration of air. On the basis of known half-lives, decay schemes and fission yields, the peaks can be assigned to the following fission products: (1) cerium-141 and cerium-144, (2) ruthenium-103, (3) zirconium-95 + niobium-95. These assignments were confirmed in this laboratory by following the decay of the photo-peaks and by carrying out radiochemical separations. Using the peaks, the listed fission products present in atmospheric dust due to nuclear test explosions can be determined quantitatively by gamma-spectrometry.

Since dust particles are inhaled and some fraction of them is retained in the respiratory tract, we have attempted to detect fission products in lungs by gamma-spectrometry. Lungs from animals and humans were ashed and the gamma-spectrum of the ashes was measured using a well-type sodium iodide (thallium) crystal. The spectrum of the ashes of a human lung is shown in Fig. 1*b*. It is apparent that the fission products detected in dust by gamma-spectrometry are also present in lung tissue. From the spectra the contents of zirconium-95 + niobium-95 and of ruthenium-103 can be calculated. (Contents of radiocerium can also be calculated, but the values are thought to be less reliable and are therefore not listed.) The values obtained from our measurements are given in Table 1.

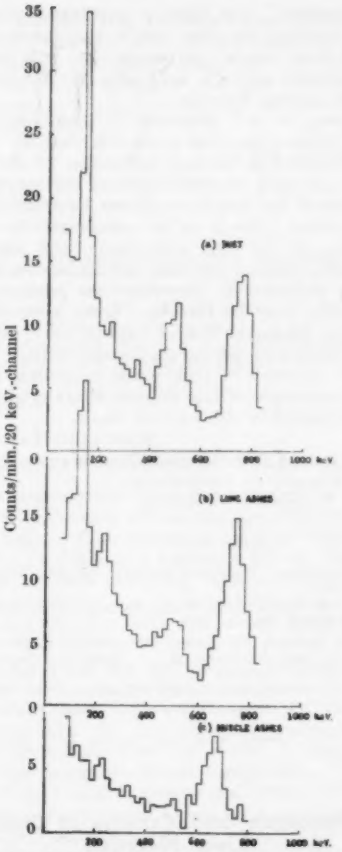

Fig. 1. Typical gamma-spectra (obtained with 20 keV.-channels, counting 16 min. in each channel; background subtracted) (*a*) Dust sample from 88 m.³ air (collected April 27, 1959; measured May 6, 1959). (*b*) Lung ashes from human *C* (measured May 26, 1959). (*c*) Muscle ashes from human *C* (measured May 27, 1959)

Table 1. Fission Products in Organs

Organ	Origin of sample	Date of death§	Weight of sample (gm.)	Weight of ashes (gm.)	Activity in sample ($\mu\mu$c.) $^{95}Zr^{f}$ + ^{95}Nb	^{103}Ru	^{137}Cs
Lung*	cow	24.2.59	4,500	32·1	1,900	280	—¶
Lung*	sheep *A*	6.4.59	310	4·1	150	30	—
Lung*	sheep *B*	6.4.59	340	4·5	240	35	—
Lung*	sheep *C*	13.4.59	320	2·9	175	45	—
Lung*	sheep *D*	13.4.59	290	5·1	140	35	—
Lung*	human *A* (50 years)‡	24.4.59	1,370	8·1	370	55	—
Lung†	human *B* (73 years)‡	12.5.59	400	1·8	155	25	—
Lung†	human *C* (60 years)‡	17.5.29	600	1·9	250	45	—
Muscle	human *B*		710	6·9	—¶	—¶	120
Muscle	human *C*		450	2·6	—	—	100
Liver	human *B*		1,350	12·9	—	—	100
Liver	human *C*		1,605	15·3	—	—	115

* Sample consisted of entire lung.
† Sample consisted of approximately half a lung.
‡ Age at death.
§ All radioactivity measurements were carried out within 5–10 days after death.
¶ Activity not detected or too small for quantitative evaluation.

Control measurements have been made on other organs (muscle, liver) from two of the humans from which lungs were obtained. The spectra of the muscle and liver ashes show only the presence of cæsium-137 (photo-peak at 662 keV.); the characteristic peaks found for dust and lung samples are not observed (Fig. 1c). (The peak due to the 1·46 MeV.-gamma radiation of potassium-40 was also observed, but is not shown in the diagram.) The potassium contents of the ashed lung and muscle tissues from one of the humans were determined by chemical analysis. From the potassium found in muscle ashes a value of the specific activity of cæsium-137 referred to potassium was obtained (84 μμc. cæsium-137 per gram potassium). (This value is somewhat higher than the range of values found by whole-body counting during 1956–57 (25–70 μμc. per gm. potassium, ref. 3) or early in 1958 (30–60 μμc. per gm. potassium, ref. 4).) Using this specific activity and the potassium content of the lung ashes it can be estimated that the activity of cæsium-137 in the lung amounts approximately to one-tenth only of the observed zirconium-95 + niobium-95 activity. This explains why cæsium-137 was not clearly detected in the spectra of lung ashes.

The amounts of radioelements found in lungs can be compared with the corresponding figures for air, as obtained from measurements on dust samples. During the past few months we have usually observed concentrations of fission products within the following ranges: 2·5–5·0 μμc. zirconium + niobium-95 per m.³ air and 0·8–1·6 μμc. ruthenium-103 per m.³ air. Thus, lungs of adult humans contain at the present time, by order of magnitude, as much fission products as a hundred cubic metres of air. It should also be noted that the activities of fission products found for the various lung samples (cow, sheep and human) agree fairly well if referred to one gram of lung tissue: for the activity of zirconium-95 + niobium-95 six values are within the range 0·39–0·54 μμc. per gram tissue and all eight values are within the range 0·27–0·74 μμc. per gram tissue; for the activity of ruthenium-103 five values are within the range 0·060–0·091 μμc. per gram tissue, and all eight values are within the range 0·04–0·14 μμc. per gram tissue.

The possibilities of using measurements of the type reported here for the study of dust retention and excretion will be discussed in a later detailed publication.

This investigation has been carried out as part of work done under a research contract with the International Atomic Energy Agency. Human lungs were put at our disposal by Prof. H. Hayek, Institute of Anatomy, University of Vienna. The dust samples were collected by Prof. J. A. Schedling, Mr. W. A. Müller and Mr. M. Sedlacek, First Physical Institute, University of Vienna.

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Incorporation of Tritium-labelled Thymidine and Tritium-labelled Deoxycytidylic Acid into Deoxyribonucleic Acid after Irradiation of the Deoxyribonucleic Acid Primer with γ-Rays

Recently, Kornberg *et al.*[1] have demonstrated that deoxyribonucleic acid formed *in vitro* by enzymatic synthesis has chemical and physical properties similar to the acid used as primer. Since exposure to radiation is known to cause chemical changes in deoxyribonucleic acid[2–4], it was thought that such changes might be reflected in the newly synthesized acid. In an attempt to study this, we have determined the ability of irradiated and non-irradiated deoxyribonucleic acid to incorporate tritium-labelled thymidine or tritium-labelled deoxycytidylic acid in the presence of the deoxyribonucleic acid-synthesizing enzyme system from regenerating rat liver.

Deoxyribonucleic acid isolated from calf thymus by the method of Kay *et al.*[5] was used in all experiments. An aqueous solution of the acid (0·4 per cent) was irradiated with various doses of γ-rays from a cobalt source at a rate of 4,000 roentgens/min. Irradiated as well as non-irradiated samples were then used as primers to study the incorporation of either labelled thymidine or labelled deoxycytidylic acid into deoxyribonucleic acid in the presence of the following compounds: deoxyadenylic acid, deoxyguanylic acid, deoxycytidylic acid, thymidine, adenosine triphosphate and the 'deoxyribonucleic acid-synthesizing enzyme system' prepared from regenerating rat liver. The assay procedure used was that of Bollum and Potter[6] except that the incubation time was 30 instead of 60 min.

The results of two series of experiments using two tritiated precursors of deoxyribonucleic acid and three radiation doses are shown in Table 1. Each value with standard deviation of the mean is based on quadruplicate or quintuplicate assays. It is apparent that the amount of labelled thymidine incorporated into deoxyribonucleic acid diminished as the radiation dose to the primer increased. In the case of labelled deoxycytidylic acid, however, the amount incorporated increased after irradiation of the deoxyribonucleic acid with 20,000 roentgens but decreased with higher doses to values approximating to those observed with non-irradiated deoxyribonucleic acid. Fig. 1 shows that the ratio of tritiated deoxycytidylic acid to thymidine incorporated into deoxyribonucleic acid was linearly related to the radiation dose under these conditions of study.

Base analysis of irradiated and non-irradiated deoxyribonucleic acid using Wyatt's paper chromatography method[7] showed that irradiation caused no significant change in the base ratio even after exposure to 200,000 roentgens. This indicates that other types of physico-chemical changes in the primer such as those involving hydrogen bonds must be responsible for our observations.

It is interesting to speculate that the changes in the capacity of irradiated deoxyribonucleic acid serving as a primer to incorporate tritiated thymidine and deoxycytidylic acid may be analogous to the altered ability of mutated genes to replicate themselves. A simple calculation serves to show that this hypothesis is not beyond the realm of possibility.

The following reasonable values can be used to calculate the mutation-rate of a gene in an irradiated living cell: (*a*) the molecular weight of deoxyribo-

Table 1. Incorporation of Tritium-labelled Thymidine and Deoxycytidylic Acid into Irradiated Deoxyribonucleic Acid

Group	Experiment 1 Radiation dose (roentgens) 0	20,000	240,000	Experiment 2 Radiation dose (roentgens) 0	20,000	120,000
Radioactivity* of tritium-labelled thymidine incorporated into deoxyribonucleic acid	159 ± 14	109 ± 15	9 ± 5	64 ± 8	48 ± 4	5 ± 1
Radioactivity* of tritium-labelled deoxycytidylic acid incorporated into deoxyribonucleic acid	41 ± 3	113 ± 25	55 ± 8	70 ± 9	113 ± 10	45 ± 6
Ratio† of incorporation of tritium-labelled deoxycytidylic acid to tritium-labelled thymidine into deoxyribonucleic acid	1·00	4·00	23·5	1·00	2·16	8·90

*Radioactivity is expressed by counts per min. per 100 μgm. of deoxyribonucleic acid.

$$\dagger \text{ Ratio} = \frac{\left(\frac{\text{Radioactivity of tritium-labelled deoxycytidylic acid in irradiated deoxyribonucleic acid sample}}{\text{Radioactivity of tritium-labelled thymidine in irradiated deoxyribonucleic acid sample}}\right)}{\left(\frac{\text{Radioactivity of tritium-labelled deoxycytidylic acid in non-irradiated deoxyribonucleic acid sample}}{\text{Radioactivity of tritium-labelled thymidine in non-irradiated deoxyribonucleic acid sample}}\right)}$$

nucleic acid is 6×10^6 (ref. 8); (*b*) one molecule of deoxyribonucleic acid contains 2×10^4 nucleotides, each of molecular weight 300; (*c*) the size of the nucleus is 5×10^{-11} cm.^{3} (ref. 9); (*d*) the deoxyribonucleic acid content per cell is 5×10^{-12} gm. (ref. 10). Assuming that one molecule of deoxyribonucleic acid corresponds to a gene, the mutation-rate in the irradiated cell would be $5{\cdot}8 \times 10^{-5}$ *G* per roentgen per nucleus per gene, where *G* equals the number of molecules affected per 100 eV. of energy absorbed. In the experiments reported in this communication, the above-mentioned ratio of 3 : 1 for the tritiated deoxycytidylic acid and thymidine incorporated into deoxyribonucleic acid irradiated with 20,000 roentgens can be used to calculate the *G* values of this system. This turns out to be 44, provided all nucleotides are equally important in the synthesis of deoxyribonucleic acid.

Substituting this value for *G* in the expression given above, the rate of molecular alteration of the irradiated deoxyribonucleic acid primer (which could be thought of as analogous to the mutation-rate in a gene) is $2{\cdot}7 \times 10^{-3}$ per roentgen per nucleus per deoxyribonucleic acid molecule. This value is much higher than the mutation-rate of 10^{-6} or 10^{-5} per gene observed in Nature. However, in other studies of irradiated deoxyribonucleic acid such as those involving its transforming activity[11], it has been shown that only one-tenth of the molecule is responsible for the activity. If the primer has a comparably small active site, the rate of alteration of irradiated deoxyribonucleic acid per roentgen would be reduced considerably. When irradiated *in vivo*, protection of deoxyribonucleic acid by other nuclear constituents would also help to reduce the difference between the rates noted in the genes of living cells and those noted under the conditions of our experiments.

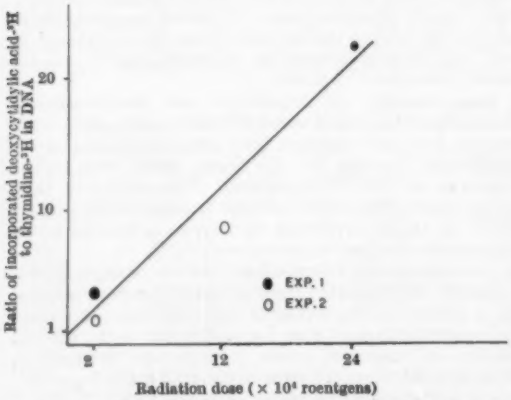

Fig. 1. Changes of ratio of tritium-labelled deoxycytidylic acid to tritium-labelled thymidine incorporated into deoxyribonucleic acid primer irradiated with various doses of radiation. The ratio defined in Table 1 has been used

Further studies on the replicating ability of irradiated deoxyribonucleic acid will be reported elsewhere.

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ARCHÆOLOGY

Spectrographic Analysis of Romano-British Pottery

Fragments of pottery are the most common signs of human occupation on most archæological sites. Their origin can often be deduced from their shapes and fabrics. There are many cases, however, where this evidence alone is ambiguous, and the work described herewith was undertaken to investigate the possibility of identifying the origin from the concentrations of various minor or trace constituents. Such variations of composition of the ceramic body would tend to reflect the variations in the clay from which it was made[1].

A large number of Romano-British mortaria were analysed spectrographically. This type of

pottery is particularly well suited to the purpose as it is very often stamped with a potter's name or mark, and therefore its place of origin can frequently be deduced from the distribution of the stamps. The rim shapes and the fabric also tend to follow regional traditions and provide additional evidence. The mortaria chosen for reference standards were either from kiln sites or were those the origin of which could be deduced from archæological evidence.

Table 1. Concentration Ranges for the Reference Elements in Mortaria regarded as Typical of Particular Sites

Origin	No. of samples	Per cent Na_2O	Per cent MgO	Per cent MnO	Per cent TiO_2	Per cent CaO
Lincoln area	3	0·23 +0·09 −0·05	0·43 +0·02 −0·01	0·01 +0·00 −0·00	1·25 +0·35 −0·15	0·64 +0·12 −0·23
Hunts. Stibbington	5	0·26 +0·05 −0·10	0·33 +0·07 −0·06	<0·01 +0·00	0·92 +0·28 −0·14	0·73 +0·08 −0·10
Nr. Doncaster. Rossington Bridge	5	0·32 +0·11 −0·07	>1·0	0·035 +0·033 −0·012	0·49 +0·11 −0·23	0·24 +0·13 −0·10
Cantley	3	0·33 +0·01 −0·01	1·0 +0·15 −0·18	0·016 +0·006 −0·004	0·55 +0·21 −0·17	0·31 +0·03 −0·05
Cheshire. Wilderspool	5	0·59 +0·22 −0·12	>2 −0·0	0·061 +0·023 −0·024	0·71 +0·24 −0·16	1·16 +0·49 −0·39
Warwickshire. Mancetter	11	0·33 +0·33 −0·19	>1·0 −0·5	0·050 +0·034 0·029	1·03 +0·37 −0·38	0·31 +0·16 −0·11
Hartshill	2	0·22 +0·05 −0·02	0·70±0·00	0·011 +0·001 −0·000	0·63 +0·09 −0·08	0·41 +0·02 −0·03
W. Midlands. Wroxeter area	10	0·21 +0·08 −0·08	0·93 +0·27 −0·21	0·017 +0·027 −0·008	0·72 +0·17 −0·25	0·32 +0·16 −0·11
Herts/Middx. Group A	3	0·14 +0·02 −0·02	0·52 +0·01 −0·00	<0·01 +0·00	0·74 +0·18 −0·14	0·60 +0·05 −0·04
Group B	3	0·22 +0·04 −0·05	0·47 +0·20 −0·15	0·018 +0·006 −0·003	0·99 +0·09 −0·08	0·17 +0·03 −0·03
Brockley Hill	7	0·15 +0·08 −0·05	0·49 +0·25 −0·18	~0·01	0·37 +0·12 −0·07	0·44 +0·16 −0·12
Radlett	3	0·15 +0·03 −0·02	0·44 +0·09 −0·14	<0·01 +0·00	0·40 +0·09 −0·14	0·34 +0·08 −0·16
E. Anglia. Colchester (Essex)	7	0·35 +0·34 −0·17	0·87 +0·43 −0·22	0·038 +0·012 −0·020	0·37 +0·11 −0·11	3·4 +1·9 −1·4
Hevingham (Norfolk)	2	0·32 +0·02 −0·03	0·80 +0·20 −0·20	0·86 +0·19 −0·19	0·82±0·02	1·56 +0·06 −0·06
Mon. Caerleon area	3	0·67±0·02	1·6±0·3	0·036 +0·030 −0·020	0·43±0·05	0·26 +0·09 −0·08
Dorset. Corfe Mullen	2	0·12 +0·02 −0·03	0·26±0·16	<0·01+0·00	0·51 +0·11 −0·12	0·18 +0·04 −0·05

The percentages given are mean values, and the maximum divergences observed are indicated above and below this figure.

Table 2. Concentration Ranges for the Reference Elements in Colour-Coated Wares regarded as Typical of Particular Sites

Origin	No. of samples	Per cent Na_2O	Per cent MgO	Per cent MnO	Per cent TiO_2	Per cent CaO
Lincs. Swanpool	3	0·33 +0·06 −0·05	>1·0	0·046 +0·043 −0·026	~5	1·1±0·0
Hunts. Chesterton	9	0·35 +0·08 −0·08	>1·0	0·013 +0·009 −0·003	1·6 +0·8 −0·8	0·82 +0·28 −0·25
Essex. Colchester	5	0·48 +0·04 −0·02	>1·0	0·081 +0·011 −0·016	2·6 +0·7 −0·7	~2·5 +0·8 −0·4
Rutland. Great Casterton	3	0·25 +0·04 −0·04	0·57 +0·11 −0·11	<0·01+0·00	2·6 +0·4 −0·2	1·07 +0·23 −0·11

The spectra were photographed in a Hilger large quartz spectrograph. $5 \pm 0{\cdot}05$ mgm. of a 1 : 1 : 1 mixture of sample/graphite/ammonium sulphate containing 1·5 per cent lithium carbonate were arced at 8–9 amp. for 30 sec. in shallow graphite cups against a flat cathode. The arc gap was kept at 4 mm.; a 3-mm. diaphragm 2·5 cm. from it helped to reduce the background. The dilution of the sample was found to improve the burning properties of these refractory materials and to reduce matrix effects[3–4]. The lithium was also useful as an internal standard in some of the experiments.

Considerable systematic regional variations in the concentrations of calcium, sodium, titanium, magnesium and manganese were observed by visual comparison with the spectra of series of mixtures of oxides of 50 elements in an ammonium sulphate base arced under similar conditions. The variations in iron, copper, zirconium, vanadium, chromium and nickel content were either small or masked by the random fluctuations from sample to sample. By means of British chemical standard samples of silica brick and firebrick, calibration curves were set up for the first group of elements. To check the internal consistency of the method in the case of manganese, calcium, and titanium, calibration curves for two lines of different intensities were used. The concentrations of the chosen elements in typical samples were averaged for each kiln area and the results are shown in Table 1. Another 80–100 samples of less certain attribution on archæological grounds were then similarly analysed and the results compared with those in Table 1. In most cases the two kinds of evidence taken together have made the kiln area virtually certain. The details of this work will be published elsewhere, together with results for further kiln-site material as they become available.

From the results it seems that the number of elements for which the quantitative analyses were carried out is sufficient to differentiate between material from well-separated regions. The differences of concentration of the various elements from region to region, when taken together, should leave little ambiguity about the source of the material when deciding between a limited number of archæological alternatives. The decision is made easier in some cases: for example, the compositions of the samples from Lincoln show high titanium, and from Colchester high calcium.

The results for some colour-coated wares, selected by Mr. G. Webster (Table 2), also show significant trends. It is impossible to determine the origin of much of this pottery on purely archæological grounds, and it is for this kind of problem that the spectrographic method may become useful. The colour-coated samples have also been analysed for potassium, gallium, strontium, barium and lead by a different technique, and the results obtained so far suggest that these data will add further criteria of the origin.

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Neutron Activation Analysis of Ancient Roman Potsherds

Thirty-two fragments of Roman samian ware have been analysed for their sodium and manganese content, using neutron activation and γ-ray spectrometry. They were chosen from at least 10 of about 30 factories at which this fine-grained pinkish-red Roman table-ware was manufactured. The body of the sherds from all factories is very similar in appearance and a chemical method was sought to differentiate between factories. Similar analyses on other types of pottery have been done by Sayre *et al.*[1,2] at Brookhaven National Laboratory, who found quantitative chemical differences in material from different places. Our results, summarized in Table 1, for 10 or more of the factories, show that this also holds in general for samian ware.

Table 1

Region	No. of factories	No. of sherds analysed	Manganese Average content*	Manganese Standard deviation	Sodium Average content*	Sodium Standard deviation
1. Italy	?	3	8	0·5	2·2	0·25
2. North-east Gaul (east of Moselle valley)	3	9	4	0·45	1·6	0·2
2 (*a*). Chémery, north-east Gaul (east of Moselle valley)	1	3	2	0·2	2·4	0·1
3. Central-south Gaul	3	11	3	0·15	0·7	0·15
4. North-east Gaul (west of Moselle valley)	2	6	3	0·1	0·6	0·1

* In arbitrary units.

50-mgm. samples from the interior of the sherds were irradiated together (so that results for different sherds would be comparable) for 4 hr. in a flux of $1{\cdot}1 \times 10^{12}$ neutrons/cm.²/sec. in *Bepo* at Harwell. The quantities of the elements present were measured in terms of the intensity of the 0·84 MeV. manganese-56 γ-ray and the 2·76 sodium-24 γ-ray, and as no standards were used the results are in arbitrary units. The reactions considered were the thermal neutron reactions manganese-55 (n,γ) manganese-56 and sodium-23 (n,γ) sodium-24, any contribution to the total sodium-24 activity from the fast neutron reaction aluminium-27 (n,α) sodium-24 on aluminium in the sherds being negligible.

Sherds tended to group regionally, and one factory at Chémery in north-east Gaul (east of the Moselle valley) was distinct from the other three in the region. That manufactured close at hand and to the west of this valley is also distinct (group 4), but on the other hand, does not differ significantly from that made in south and central Gaul. Whatever the reason for these differences they are of archæological interest, and suggest a means for locating the region of origin of samian sherds which cannot be placed stylistically.

We gratefully acknowledge the help of Mrs. E. Heckmann, who initiated the work and developed the gamma-ray spectrometer used.

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the History of Art, Oxford. Dec. 3.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, January 18

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5 p.m.—Mr. Peter Haggett: "Landuse in the Serra do Mar, Brazil".

UNIVERSITY OF LONDON (in the Meyerstein Lecture Theatre, Westminster Medical School, Horseferry Road, London, S.W.1), at 5.15 p.m.—Prof. V. M. Trikojus (University of Melbourne): "Some Aspects of Thyroid Biochemistry".*

BRITISH SOCIETY FOR THE PHILOSOPHY OF SCIENCE (in the Eugenics Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. K. R. Popper: "An Indeterminist's View of the Physical World" (Presidential Address).

SOCIETY OF CHEMICAL INDUSTRY, PESTICIDES GROUP (at 14 Belgrave Square, London, S.W.1), at 5.30 p.m.—Dr. L. Crombie: "Lipid *Iso*butylamides".

ROYAL INSTITUTE OF CHEMISTRY (at Enfield Technical College, Queensway, Ponders End, Enfield, Middlesex), at 7.30 p.m.—Dr. D. K. Hill: "Recent Advances in Glass Technology".

Monday, January 18—Friday, January 22

PHYSICAL SOCIETY (at the Royal Horticultural Society's Halls, Westminster, London, S.W.1)—44th Exhibition of Scientific Instruments and Apparatus.

Tuesday, January 19

UNIVERSITY COLLEGE (in the Anatomy Theatre, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. A. Comfort: "Darwin and Freud".*

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Superseding the Ratio Arms in A.C. Bridges", opened by Mr. C. G. Mayo.

UNIVERSITY COLLEGE (in the Physics Theatre, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. Alonzo Church (Princeton University): "Logic and Meaning".* (First of four Shearman Memorial Lectures. Further Lectures on January 21, 26 and 28.)

PLASTICS INSTITUTE (at the Wellcome Building, 183–193 Euston Road, London, N.W.1) at 6.30 p.m.—Dr. G. Swift: "Plastics in the Packaging Industry".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. J. Seddon: "Some Aerodynamic Problems of Engine Installation".

PHARMACEUTICAL SOCIETY OF GREAT BRITAIN (at 17 Bloomsbury Square, London, W.C.1), at 7.30 p.m.—Dr. N. J. Harper: "A Three Dimensional Approach to Drug Design".

Wednesday, January 20

PHYSICAL SOCIETY, ACOUSTICS GROUP (in the Physics Department, Imperial College, Imperial Institute Road, London, S.W.7), at 2.30 p.m.—Symposium on "Vibrations".

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 5 p.m.—Monsieur N. Gerbier (Paris): "Experimental Studies of Wave-form Clouds"; Dr. E. Palm (Oslo): "Recent Developments in Theoretical Treatment of Mountain Waves".

ROYAL STATISTICAL SOCIETY (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.15 p.m.—Mr. K. F. Glover: "Statistics of the Transport of Goods by Road".

EUGENICS SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Mr. Robert H. S. Robertson: "The Output of Scientists in Scotland—1600 to 1950".

INSTITUTE OF PHYSICS (at 47 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. H. J. Dunster: "Health Physics".

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "One Approach to Generalized Machine Analysis", opened by Mr. G. J. Henderson.

ROYAL INSTITUTE OF CHEMISTRY (joint meeting with the BRITISH SOCIETY FOR THE HISTORY OF SCIENCE, at the Science Museum, Exhibition Road, South Kensington, London, S.W.7), at 6.30 p.m.—Mr. F. Greenaway: "Thirty Centuries of Assaying".

OIL AND COLOUR CHEMISTS' ASSOCIATION, LONDON SECTION (at the Royal Society of Tropical Medicine and Hygiene, Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. G. L. Cooper: "An Appreciation of Work Study", followed by a film "Introducing Work Study".

SOCIETY FOR ANALYTICAL CHEMISTRY, BIOLOGICAL METHODS GROUP (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Prof. L. Garrod: "Antibiotic Assays in Body Fluids".

SOCIETY FOR PSYCHICAL RESEARCH (at Kensington Town Hall, Kensington High Street, London, W.8), at 7 p.m.—Dr. S. G. Soal: "The Jones Boys Experiments—The Case Against Cheating".

Wednesday, January 20—Thursday, January 21

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (in co-operation with the BRITISH COMPUTER SOCIETY, at Savoy Place, London, W.C.2)—Discussion Meetings on "Reliability and Maintenance of Digital Computer Systems—Managerial and Engineering Aspects".

Thursday, January 21

INSTITUTION OF MINING AND METALLURGY (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Mr. J. H. Harris: "Serial Gravity Concentration—a New Tool in Mineral Processing"; Mr. P. D. R. Maltby: "Use of Moving Bed Ion Exchange in the Recovery of Uranium at Can-Met Explorations, Ltd., Blind River, Ontario".

LONDON MATHEMATICAL SOCIETY (at the Royal Astronomical Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. S. J. Taylor: "Properties of the Pólya Random Walk".

INSTITUTE OF INFORMATION SCIENTISTS (at Caxton Hall, Caxton Street, London, S.W.1), at 6 p.m.—Mr. R. L. Brown: "The Critical Technical Review and Its Contribution to Progress".

INSTITUTION OF MECHANICAL ENGINEERS, HYDRAULICS GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "The Value of Hydraulics Research in Mechanical Engineering".

SOCIETY OF CHEMICAL INDUSTRY, ROAD AND BUILDING MATERIALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6 p.m.—Mr. D. B. Honeyborne: "Plastics for Building".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Symposium on "Arthropod-borne Diseases"; Dr. C. E. Gordon Smith: "Factors in the Past and Future Evolution of the Arborviruses"; Mr. P. F. Mattingly: "Ecological Aspects of the Evolution of Mosquito-borne Virus Diseases"; Prof. C. D. Darlington: "Origin and Evolution of Viruses".

Saturday, January 23

BIOCHEMICAL SOCIETY (in the Department of Biochemistry, London Hospital Medical College, Turner Street, London, E.1), at 10 a.m.—390th Meeting.

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. Philip Sumner: "The Wheel in the History of Transport".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURER IN THE DEPARTMENT OF SOCIOLOGY—The Registrar, The University, Leicester (January 30).

CHIEF CATALOGUER (graduate with good cataloguing experience, preferably of the Library of Congress scheme)—The Registrar, The University, Nottingham (January 30).

LECTURER (honours graduate in physics or chemistry) IN POLYMER CHEMISTRY in the Department of Textile Chemistry—The Registrar, The Manchester College of Science and Technology, Manchester 1 (January 30).

LECTURER IN PHYSICS AND/OR CHEMISTRY—The Principal, Borough Road College, Isleworth, Middlesex (January 30).

RESEARCH ASSISTANT IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, The University, Liverpool, quoting Ref. CV/T (January 30).

SCIENTIFIC OFFICER (male, with an honours degree), for genetical and cytological research—The Secretary, Plant Breeding Institute, Trumpington, Cambridge (January 30).

ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Sheffield (January 31).

BOOTS MEDICAL RESEARCH FELLOW (medically qualified), to undertake research in one of the following fields: Pharmacology, Therapeutics, Pathology, Bacteriology, Medicine, Surgery, or Obstetrics and Gynæcology—The Registrar, The University, Sheffield (January 31).

LECTURER IN BACTERIOLOGY—The Registrar, The University, Sheffield (January 31).

ASSISTANT LECTURER IN BIOCHEMISTRY—The Registrar, The University, Sheffield (January 31).

ASSISTANT LECTURER IN MATHEMATICS—The Registrar, University College of North Staffordshire, Keele, Staffordshire (February 1).

LECTURER/SENIOR LECTURER IN MECHANICAL ENGINEERING at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 2).

SENIOR LECTURER or LECTURER, and a LECTURER IN THE DEPARTMENT OF MECHANICAL ENGINEERING, University of Western Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, February 8).

CHAIR OF DERMATOLOGY at the Institute of Dermatology—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 10).

READER IN LARYNGOLOGY at the Institute of Laryngology and Otology—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 11).

EXPERIMENTAL OFFICER/ASSISTANT EXPERIMENTAL OFFICER (electronic engineer with experience of pulse techniques) at the National Physical Laboratory (Metallurgy Division), Teddington, Middlesex, for the upkeep and development of the following equipment: nucleonic counters, X-ray detectors, spectrographic source units, automatic timers, voltage regulators, mass spectrometers, and

wide band amplifiers—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting Ref. D.633/9A (February 12).

Lecturer in Physics, and a Laboratory Superintendent in the Department of Physics, Fourah Bay College, Sierra Leone (University of Durham)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (February 12).

Assistant Lecturer or Lecturer (with a degree in pure science) in Agricultural Zoology at Canterbury Agricultural College, University of New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 15).

Chair of Organic Chemistry at Queen Mary College—The Academic Registrar, University of London, Senate House, London, W.C.1 (February 15).

Senior Lecturer and a Lecturer in Physics at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 29).

Senior Lecturer in Agricultural Economics at Canterbury Agricultural College, University of New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, February 29).

Senior Lecturer or Lecturer in Education at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1, or from the Registrar of the University (New Zealand and London, February 29).

Assistant Tutor or Tutor in Pharmaceutics; an Assistant Tutor or Tutor in Pharmaceutical Chemistry; an Assistant Tutor or Tutor in Pharmacognosy; and an Assistant Tutor in Pharmacology at the New Zealand School of Pharmacy—The High Commissioner for New Zealand, 415 Strand, London, W.C.2 (March 1).

Biochemist or Microbiologist (with an honours degree), to work in the field of bacterial metabolism—The Secretary, Medical Research Council of Ireland, 50 Merrion Square, Dublin (March 1).

Peter Nicol Russell Chair of Mechanical Engineering at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, March 15).

Assistant Physicist (with an honours degree in physics and postgraduate experience in radiation or nuclear physics) in the Department of Physics Applied to Medicine, for duties concerned mainly with assisting in the organization of practical work, demonstrations and teaching aids for postgraduate courses for the M.Sc. degree in Radiation Physics, and other postgraduate courses in radiological physics and protection—The Secretary, Department of Physics Applied to Medicine, Middlesex Hospital Medical School, London, W.1.

Associate Professor or Assistant Professor (theoretical physicist, preferably with experience and main interest in the field of nuclear theory), to teach courses at the senior and postgraduate levels and to carry on research—The Head, Department of Mathematical Physics, University of Manitoba, Winnipeg, Canada.

Biologist (with a good degree in physiology or zoology and considerable research experience, preferably with spermatozoa); and a Biochemist (with a good degree in biochemistry or chemistry, and research experience with polysaccharides or proteins, preferably involving work with animals), to join a small research team investigating cervical mucus, with particular reference to problems of fertility—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 59/17.

Biologist (with a strong leaning towards botany, and capable of teaching biology to *A* level and botany to *S* level); and a Chemist (capable of teaching the subject to *A* level)—The Head Master, Westminster School, Dean's Yard, London, S.W.1.

Chief Technician (trained in hæmatology, able to supervise staff and plan systematic experimental work)—The Personnel Manager, Jewish General Hospital, 3755 Cote Street, Catherine Road, Montreal, Quebec, Canada.

Graduate Assistants (qualified to pursue M.Sc. and Ph.D. work) in Chemistry—The Chairman, Department of Chemistry, McMaster University, Hamilton, Ontario, Canada.

Indexer (female, preferably graduate)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, near Maidenhead, Berks.

Instructor or Assistant Professor; Postdoctorate Fellows; and Scholars in the Department of Physics—The Head, Department of Physics, University of British Columbia, Vancouver, B.C., Canada.

Isotope Technician (preferably with a knowledge of biochemistry or electronics), for a specialized laboratory dealing with the diagnosis, treatment and research in thyroid disorders—Hospital Secretary, New End Hospital, Hampstead, London, N.W.3.

Laboratory Technician (A.I.M.L.T. or B.Sc.), for routine hospital biochemistry—The Secretary, Group 9 Pathological Laboratory, 6 Rickmansworth Road, Watford.

Lecturer (with an honours degree in mathematics or statistics, with teaching and research or industrial experience involving the application of mathematics to scientific and engineering problems) in Mathematics—The Registrar, College of Technology, Ashley Down, Bristol 7.

Professor's Secretary (preferably graduate, especially botany or horticulture), to work at the University of Reading Horticultural Research Laboratories—The Assistant Bursar, The University, Reading.

Research Assistant (with some previous laboratory or animal experience), for work on the biochemistry of the eye—The Secretary, University College Hospital Medical School, University Street, London, W.C.1.

Research Fellow in the Department of Medical Physics, for work concerned with the physical problems arising from the use of ionizing radiations in medicine—The Secretary, Royal Free Hospital School of Medicine (University of London), 8 Hunter Street, London, W.C.1.

Scientific Officer (with a first- or second-class honours degree in physics or equivalent) at the National Physical Laboratory (Standards Division), Teddington, Middlesex, to apply modern techniques, for example, optical interference, to precision measurement in first place of quantities, such as length and pressure, and later of fundamental physical quantities and constants—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting A.577/9A.

Senior Pharmacist at the London Chest Hospital—The House Governor, London Chest Hospital, London, E.2.

Technical Assistant (preferably with G.C.E. "A" level, or better, in physics, chemistry and biology), for research in cell structure and nucleic acids, using the electron microscope, etc.—The Director, M.R.C. Biophysics Research Unit, King's College, Strand, London, W.C.2.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

London and Home Counties Regional Advisory Council for Technological Education. Engineering Education in the Region. Seventh edition. Pp. 36. (London: London and Home Counties Regional Advisory Council for Technological Education, 1959.) 3*s.* 6*d.* [3011

Ciba (A.R.L.), Ltd. Technical Notes, No. 203 (November, 1959): 'Redux' for Bonding Brake Liners. Pp. 8. (Duxford: Ciba (A.R.L.), Ltd., 1959.) [3011

Committee on Science and Freedom. Bulletin No. 13: The Scholar and Society—Report on a Study Group held in Tunis, April, 1959. Pp. 144. (Manchester: Committee on Science and Freedom, 818, Wilmslow Road, 1959.) [3011

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World Health Organization. Library News, Vol. 12, Supplement No. 2: Current Indexing and Abstracting Periodicals in the Medical and Biological Sciences—an Annotated List. Second edition. Pp. 85. (Geneva: World Health Organization, 1959.) [3011

Académie Royale de Belgique. Classe des Sciences. Mémoires, Tome 31, Fascicule 6: Flexion et Extension des Plaques d'Epaisseur Modérée. Par Prof. B. Fraeijs de Veubeke. (Bruxelles: Académie Royale de Belgique, 1959.) [3011

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The Regional Research Centre of the British Caribbean at The Imperial College of Tropical Agriculture, Trinidad, W.I. Soil and Land-Use Surveys No. 6: British Guiana. 1: The Rupununi Savannas (continued). 2: The Intermediate Savannas. 3: General Remarks. By J. Stark, G. K. Rutherford, J. Spector and T. A. Jones. Pp. 24. (St. Augustine, Trinidad: Imperial College of Tropical Agriculture, 1959.) 15*s.* [3011

Universidade de São Paulo. Boletim do Instituto Oceanografico. Tomo VIII, Fasc. 1 e 2. Pp. 261. Contribuicoes Avulsas do Instituto Oceanografico. No. 1, 1957: *Coleridia kathetostoma* n. sp., da fam. *Dactyloscopidæ* (Pisces-Perciformes.) By J. de Paiva Carvalho. Pp. 5+1 plate. No. 2, 1957: Istituto de Biologia Maritima das Caraibas: Fundacao e Patrocinador. Pp. 5. No. 1, 1958: Red para Fitoplancton con copo Intercambiable. Pp. 5+2 plates. (São Paulo: Universidade de São Paulo, Instituto Oceanografico, 1957 and 1958.) [3011

Instituto Ecuatoriano de Ciencias Naturales, Quito, Ecuador. Contribucion No. 29: Los Manglares del Ecuador. Por Dr. M. Acosta-Solis. Pp. 82. (Quito: Instituto Ecuatoriano de Ciencias Naturales, 1959.) [3011

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